

# **Examination of Sexual Assault Cases**

# 1 Purpose

The purpose of this procedure is to describe those procedures required for the examination of sexual assault cases by Evidence Recovery scientists and technicians in Forensic DNA Analysis, in addition to those described in QIS document 17142 (Examination of Items).

# 2 Scope

This procedure applies to all Forensic DNA Analysis staff that examine or interpret examinations of evidentiary items. This standard operating procedure is in conjunction with individual methods for particular screening tests. Interpretations and limitations of reporting are to be found in each method.

### 3 Definitions

- Refer to QIS document 23849 (Common DNA Analysis Terms and Acronyms) for a comprehensive list of abbreviations.
- All references to microscopy, refer to QIS document 17189 (Examination For & Of Spermatozoa)
- All references to Acid Phosphatase (AP), refer to QIS document 17186 (The Acid Phosphatase Screening Test for Seminal Stains)
- All references to Phadebas, refer to QIS document 17193 (Phadebas Test For Saliva)
- All references to Tetramethylbenzidine, refer to QIS document 17190 (Tetramethylbenzidine Screening Test for Blood)
- All references to p-30, refer to QIS document 17185 (Detection of Azoospermic Semen in Casework Samples)
- A semen negative item is an item which has either tested negative for spermatozoa microscopically and tested negative for acid phosphatase; or tested negative for spermatozoa microscopically, tested positive for acid phosphatase and tested negative for P30.

# 4 General Principles

Refer to the general principles contained in QIS document 17142 (Examination of Items).

# 4.1 Examination Strategies

An examination strategy must be prepared for all SAIKs which are examined. These are recorded under the specimen notes of the exhibit barcode. This strategy must include:

- · For each item to be examined, what biological fluid is to be screened for
- Items which require no further action
- Items which may only require examination pending presumptive/screening results
- Sample submission strategies (i.e. extraction type, pooling, retain supernatant for Phadebas testing etc).

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The examination strategy must be reviewed by a scientist competent to perform the examinations contained in the strategy.

The following are general principles which are used to develop examination strategies for SAIKs, however these principles must be considered within the context of the case history:

- Female SAIKs which are semen negative, any external intimate swabs are submitted for cells (i.e. vulval and perianal)
- Where the complainant is a minor or has an intellectual impairment which may mean that the provided case history is unreliable, all possible offence scenarios are considered.
- Where the complainant is an adult who has lost consciousness, has impaired memory or has consumed alcohol or drugs prior to or during the offence which may impact on memory, all possible offence scenarios are considered.
- Consider previous intercourse with same or different partner, prior to the offence.
   For digital only female complainant cases with prior intercourse, submit external swabs for DLYS with no testing. For male SAIK swabs, consider submitting penile swabs for DLYS where previous intercourse with another partner has occurred.
- Consider the number of offenders for male SAIKs consider submitting penile swabs for DLYS (with no testing) to separate epithelial and spermatozoa.
- For child complainants, treat all vaginal swabs as external swabs for semen or cells.
- Samples taken from areas of biting, licking or kissing (or other oral contact) are submitted for CSUP. This does not include swabs taken from the mouth (internal or external), anal and vaginal areas which may give false positive results.

# 5 Examination

The general examination procedures documented in QIS document 17142 (Examination of Items) apply to the examination of sexual cases.

# 6 Specific Examination Strategies

Refer to Section 6.2 of QIS document 17189 (Examination for and of Spermatozoa) for procedures relating to making a suspension and preparing, staining and reading microscope slides.

# 6.1 Sexual Assault Investigation Kits

Appendix 1 describes the workflow for presumptive/screening testing of SAIKs. Before commencing the examination of a SAIK an examination strategy must be devised and reviewed in accordance with Section 4.1 of this document.

If there are issues related to the collection or documentation of a SAIK this must be fed back to the relevant FMO or FNE using the SAIK issues log. Examples of issues may include:

- Serum coated, charcoal swabs or other unsuitable swabs/media are submitted (these should tested regardless of the swab type or media and a specimen note must be added to Auslab)
- Insufficient case history
- Labelling issues/inconsistencies
- Smears have been prepared by the FMO/FNE

If a smear has not been received, one will need to be made and tested, refer to QIS document 17189 (Examination for & of Spermatozoa - Section 6).

The following principles should be applied to the submission of SAIK samples:



- Where an amount of spermatozoa which is considered likely to give a DNA profile (i.e. 3+ or more) are seen on multiple swabs from the same internal location (e.g. vagina), and there is no history of sexual contact with another person within the previous seven days or multiple offenders, then only one of these swabs should be submitted for full analysis. When selecting which swab to submit for testing, preference should be given to the highest internal swab (i.e. submit a high vaginal swab over a low vaginal swab). All of the swabs referred to should have the same or very similar micro (e.g. some 3+ some 4+)" Where swabs have different micro results (e.g. some 1+ some 3+), then submit all. Other swabs which would otherwise be submitted for full testing based on their presumptive/screening test results must be submitted but with a 'POLD' test code rather than 9plex/Xplex. These samples are then stored in the black box labelled 'SAIK swabs on hold'. This enables the Case Manager to view the results of the first swab, before assessing whether additional samples require processing.
- Submission of swabs for cells (where presumptive and screening tests are negative for semen and spermatozoa) should be considered based on the case history. If more than several days have passed since the offence, it may be unlikely that foreign DNA will be located, particularly if the subject person has bathed. Consult with the Senior Scientist for direction in these matters, however the following scenarios would justify the submission of samples for cells:
  - Child complainant
  - Complainant with mental impairment, or other impairment which may influence reliability of provided offence history
  - Complainant with loss of consciousness or drug/alcohol use which has impaired their recollection of events
  - Other circumstances as deemed appropriate by the QPS or Senior Scientist.

# 6.2 Acid Phosphatase (AP) Positive Fabrics and AP paper

Appendix 2 describes the workflow to be used for presumptive/screening testing of AP positive fabrics.

AP positive fabrics are submitted by QPS. The AP positive area should be clearly marked on the fabric. If the fabric is not marked then the entire sample should be tested, including both sides of fabric.

AP positive fabrics should be submitted with sufficient additional area surrounding the circled AP positive area to enable the examining scientist to safely hold the fabric if/when taking a scraping. Where insufficient additional area has been provided a FERRO should be created so that it may be fed back to the QPS.

Where a large piece of AP positive fabric is to be tested, divide the item into sections and test each section separately. If spermatozoa are located microscopically on one section, but are not located on other sections, P30 testing is not required to be performed on all negative sections. All sections are submitted for analysis as DLYS.

If semen is not detected there is no further action and the item is returned.

Generally fabrics are scraped or excised (extreme care to be taken to stop needle stick injuries).

At times, the paper used by QPS to test for the presence of AP will also be sent for analysis. These are items should only be examined if specifically requested by QPS. For these items, microscopy for spermatozoa should be conducted, but not p30 testing.

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# 6.3 Semen in-tubes

Appendices 2 and 3 describes the workflow for presumptive/screening testing of semen in tubes based on whether the QPS have conducted AP testing. If semen in tubes are stored in an in tube registration box, they must be transferred to an items box so that they can be examined by a scientist.

### 6.4 Condoms

Appendix 3 describes the workflow for presumptive/screening testing of Condoms.

When a condom is received it should be described in terms of "O/S surface as received" and "I/S surface as received". Describe any fluid that may be present on or within the condom. Describe length and diameter, colour, patterning and translucency of condom.

Take one wet and one dry swab from the O/S and I/S surfaces of the condom. Sample and combine I/S wet and dry swabs into one tube and O/S wet and dry swabs into another tube. N.B. When sampling the swabs, to ensure that there is not excess substrate submitted, sample the entire of the wet swab, but only the outer layer of the dry swab.

# 6.5 Sanitary Pads and Tampons

Appendix 4 describes the workflow to be used for presumptive/screening testing of sanitary pads and tampons.

Sanitary pads are AP tested on the side worn in contact with the skin.

Tampons are cut through the middle and splayed out. The outer sides of the tampon are then AP tested.

### 6.6 Post Mortem Samples

Appendix 5 describes the workflow for presumptive/screening testing of Post Mortem samples.

The examining scientist assigns an EXH barcode to the PM samples as a whole, which is passed onto QPS DNA results management (DRMU). All other samples submitted will be subsamples of the PM samples EXH (as per SAIK submissions).

The receipt under which the samples are submitted usually has an associated Coronial case number. Before any subsamples are registered this Coronial case number needs to be changed to the associated QP number by an AUSLAB Corrections Officer. If subsamples are registered under a Coronial case number the EXH lines will not be transmitted to QPS

PM samples may include sexual assault swabs and/or slides (high vaginal, low vaginal, vulval etc), pubic hair, head hair, fingernail clippings or scrapings.

Intimate sexual assault swabs which are semen positive are submitted for DLYS. Intimate sexual assault swabs which are semen negative are submitted for cells.

Sometimes the fingernail clippings include a portion of tissue or part of the finger. In this case a moistened swab can be used to sample potential foreign DNA from the underside of the nail, taking care not to sample the deceased person's tissue (i.e. targeting the distal end of the nail).



When PM samples are complete, send an E-mail to the Senior Scientist with the EXH barcode so that that information can be passed onto DRMU to facilitate electronic transfer of results from AUSLAB to the Forensic Register.

# 6.7 Clothing and Bedsheets

For large items, an examination strategy should be formulated based on the case history and if necessary consultation with the QPS. This must be recorded in the UR notes for the case.

If the case history suggests that the item has been washed then it may be necessary to perform microscopy only considering the water soluble nature of Acid Phosphatase and P30. Use the case history and if necessary communicate with the investigating officer to establish an area to target.

# 6.8 Wet and Dry swabs – QPS submitted

When wet and dry swabs are received from the same site (e.g. in a SAIK, or from one item) submit each of the swabs separately.

# 6.9 Multiple Presumptive/Screening Tests

Consideration should be given to the order in which screening tests are conducted based on the type of tests to be performed and the conservation of sample on the item. Where both AP and Phadebas screening tests are required, Phadebas the exhibit first (using commercial paper). Once the Phadebas test is complete the Phadebas paper can be sprayed with AP reagent.

# 6.10 Analytical Slides

If reading of differential slides is requested by a case scientist, retrieve slides from storage box and put a borrowed comment in AUSLAB. Stain slides and perform microscopy.

Read slides and fill out QIS document 17037 (Microscopy of Smears)

**N.B.** Old slides- DLYS step 10 slide may have both sperm and epithelial cells, whereas step 22 slides may have sperm only. Currently only one slide is made- should have sperm only.

Return slides and add a returned comment in AUSLAB.

### 6.11 Penile Swabs

The presence of spermatozoa on penile swabs is not unexpected. These swabs are generally submitted for Cells, however where the case history indicates multiple offenders, or previous sexual contact, they should be submitted for DLYS. Appendix 1 describes the workflow to be used for presumptive/screening testing of SAIK swabs.

# 6.12 Lubricant Testing

If an item is required for lubricant testing consult with Forensic Chemistry before any examinations are conducted

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# 7 Associated Documentation

QIS: 16004 QIS: 17033 QIS: 17034 QIS: 17117 QIS: 17119 QIS: 17135 QIS: 17140 QIS: 17185 QIS: 17186 QIS: 17189 QIS: 17190 QIS: 17190 QIS: 17193 QIS: 22846 QIS: 22857 QIS: 22857 QIS: 23008 QIS: 23014 QIS: 23014 QIS: 23055 QIS: 23849	AUSLAB Users Manual – DNA Analysis General Examination Record (Unruled) General Examination Record (Ruled) Procedure for Case Management Procedure for Release of Results Handling and Sampling of Syringes and Needles Procedure for the Identification and Examination of Hairs Detection of Azoospermic Semen in Casework Samples The Acid Phosphatase Screening Test for Seminal Stains Examination For & Of Spermatozoa Tetramethylbenzidine Screening Test for Blood Phadebas Test for Saliva Digital imaging in DNA Analysis General Swab Exam Record Anti Contamination Procedure Forensic DNA Analysis Outer Packaging Record Explanations of EXR/EXHs Cigarette Butt General Examination Record General Examination Record Common Forensic DNA Analysis terms and Acronyms
QIS: 23849 QIS: 23898 QIS: 26071 QIS: 31286	Common Forensic DNA Analysis terms and Acronyms SAIK Details Record Examination of in-tube samples SAIK form no semen testing

# 8 Amendment History

Version	Date	Author/s	Amendments
1	23/10/2013	L Ryan	Document created (content split
		A Houlding	from Examination of Items)
		J Seymour-Murray	
2	05/12/13	A Houlding	Update for XPlex
3	03/11/2014	A Houlding	New template, 6.2 title changed, header changed, added POLD test code for SAIK samples on hold, also apply the SAIK on hold procedure to samples with a micro result of 2+ (changed from 1+), fixed hyperlinks Changed wet and dry swabs to be submitted separately. Formatted flowcharts. Added lubricant testing section
4	24/08/2016	A McNevin	Section 4.1 and 6.2 updated other minor formatting adjustments

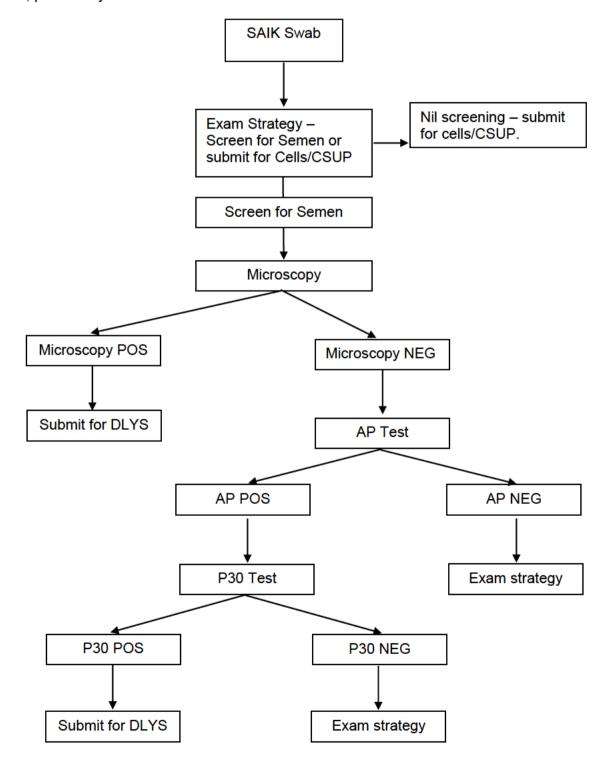
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# 9 Appendices

# 9.1 Appendix 1: SAIK Examination Workflow

This workflow is intended to demonstrate the testing of one sample from a SAIK. The submission of samples should be considered within the context of the Examination Strategy, taking into consideration the case history as well as the presumptive and screening results of other SAIK swabs, particularly those from the same location.

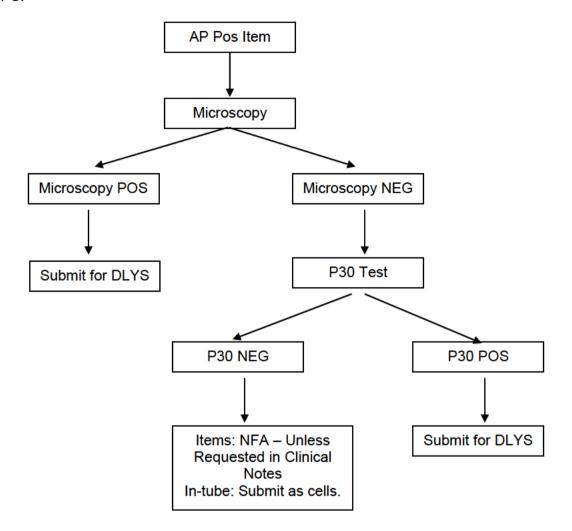


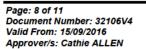




# 9.2 Appendix 2: QPS AP Tested Items (including Semen in-tubes) Workflow

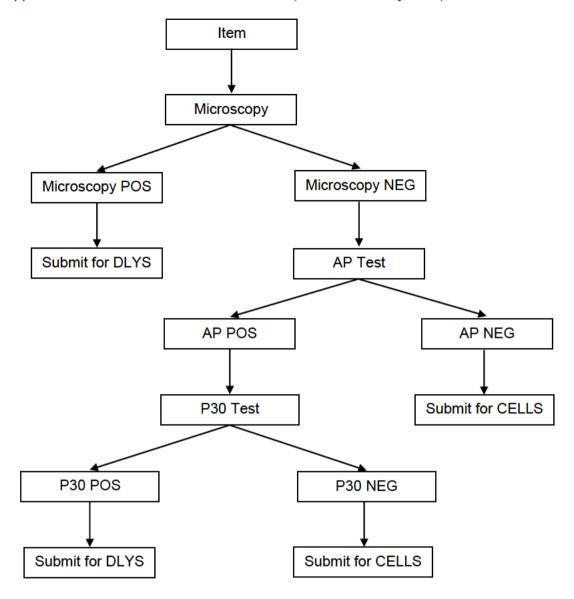
This workflow is to be used for all items which have previously tested positive using the AP test by the QPS.





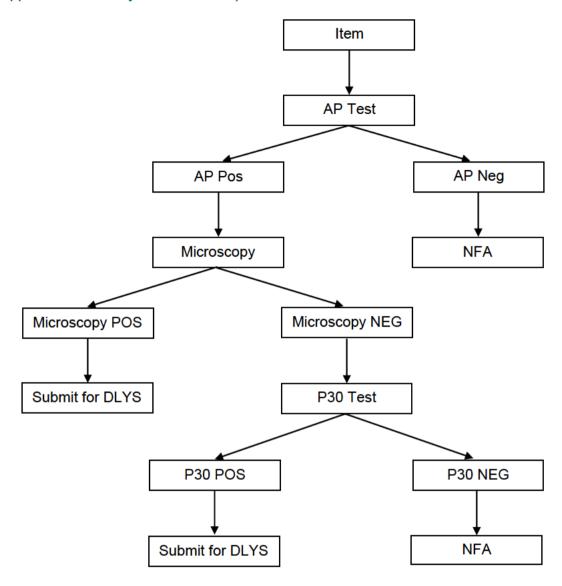


# 9.3 Appendix 3: Condoms and Semen in Tubes (not AP tested by QPS) Workflow





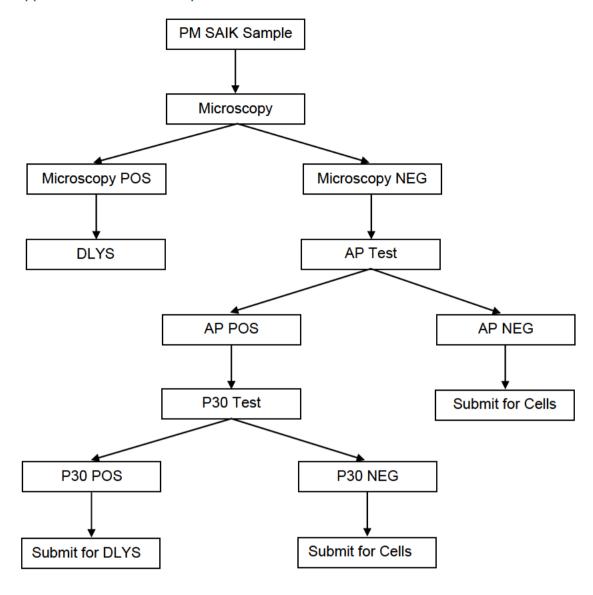
# 9.4 Appendix 4: Sanitary Pads and Tampons Workflow



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# 9.5 Appendix 5: PM SAIK Samples Workflow







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# **Examination of items**

# 1 Purpose

The purpose of this procedure is to describe the procedures for the examination of evidentiary items by Evidence Recovery scientists and technicians in Forensic DNA Analysis.

# 2 Scope

This procedure applies to all Forensic DNA Analysis staff that examine or interpret examinations of evidentiary items. This standard operating procedure is an adjunct to individual methods for particular screening tests. Interpretations and limitations of reporting are to be found in each method.

# 3 Definitions

Refer to QIS document 23849 (Common Forensic DNA Analysis Terms and Acronyms) for a comprehensive list of abbreviations.

9PLEX: Test code used for submission of samples for Profiler Plus testing XPLEX: Test code used for submission of samples for PowerPlex 21, PowerPlex Fusion or Globalfiler

Dual analysis: The term used for the examination of an exhibit by two or more forensic sections (e.g. Forensic DNA Analysis and Chemistry).

# 4 General Principles

# 4.1 Anti-contamination procedures

QIS document 22857 (Anti-contamination Procedure) describes the anti-contamination procedures for the examination of items, which must be adhered to at all times.

NOTE: Examination bench 15 must be used for reference samples, and this examination bench is reserved solely for this purpose. All other examination benches are used for crime scene samples.

# 4.2 Continuity

Continuity is the ability to demonstrate and account for the movements and ownership of an item, meaning that at any point between when the exhibit is seized through to when the exhibit is produced in court or destroyed, its location and all persons who have come in contact with the exhibit can be determined. This provides evidence that the exhibit has not had the opportunity to be tampered with, or has not come in direct contact with other exhibits. Refer to QIS document 14077 (FSS- Legal Analysis).

When moving an exhibit or case file the physical movement must be recorded electronically in AUSLAB using the transfer function (for exhibits or case files already with a physical

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location) or using the add or fill functions (for exhibits or case files without a current physical location).

In addition to recording the physical location of exhibits and case files, continuity also includes:

- Recording exhibit packaging details, including seals.
- Examination notes
- Use unique identifying numbers or barcodes for exhibits and sub-samples.
- Maintaining custody and security of exhibits at all times. Only items which are drying should be left in the laboratory overnight. All other items must be returned to the exhibit room or freezer.

**NOTE:** Staff with three initials must use three initials at all times when signing, so as to distinguish staff. Refer to QIS document 17088 (Procedure for recording handwriting specimens in Forensic DNA Analysis).

# 4.3 Forensic Relationship

The Forensic Relationship field is provided by QPS to indicate the relationship between the exhibit and the case, and where the exhibit appears to have originated from. An exhibit may have one or more forensic relationships assigned to it.

The Forensic Relationship of an exhibit can be viewed in two ways:

- In the SF9 Summary Page of a case, listed in the "Relation" column.
- On the EXR/EXH page the exhibit, listed in the "For Relationship" field.

### Definitions:

- N: No further work (All work must be ceased for items with this forensic relationship)
- S: Item/sample is believed to have originated from the suspect
- V: Item/sample is believed to have originated from the victim
- E: Item/sample is from a known source, to be used as an elimination sample
- X: Item/sample is has been found/originated from the point of entry/exit
- W: Item/sample is believed to have come from/been used as a weapon
- A: This item sample has been identified as a key sample of interest and is preferred to be sampled due to admission/ intelligence value

# 4.4 Priority

The QPS will designate a priority for a case and for exhibits (which may be different). A case/sample may be given the following priorities:

- Priority 1 (Urgent): Samples specifically approved by the QPS for processing in 3-5 day turn around. Samples may only be processed as Priority 1 with the approval of the Senior Scientist, Team Leader or Managing Scientist.
   Samples identified as needing to be processed before routine samples, due to an identified specific issue e.g. pending court date for case
- Priority 2 (High): Allocated based on crime code and generally used for crimes against a person.
- Priority 3 (Medium): Allocated based on crime code and generally used for crimes not against a person (i.e. property crime).



- Priority 6 (Cease work): Used to designate that a sample no longer requires processing and all work is to be ceased.
- Priority 4 and Priority 5 have been retained for legacy samples from old cases that
  are yet to be processed. If any of these samples are identified as requiring
  processing, they are to be changed to an appropriate higher priority based on the
  case type and other specific information to the case.

The priority of a sample/case may change at any stage and should be reviewed when determining testing or re-testing requirements.

### 4.5 Clinical notes

The QPS can enter examination strategies, or other information to guide the examination by Forensic DNA Analysis in Exhibit Notes field in the Forensic Register, which electronically populates the Clinical Notes field in AUSLAB.

# 4.6 Dual Analysis

Dual analyses must be completed in the Evidence Recovery laboratory as this location has the optimal environmental conditions for DNA sampling.

Exhibits which are to be transferred to the custody of Forensic DNA Analysis must be receipted as per normal receipting arrangements through the Property Point. Where the item is not transferred to Forensic DNA Analysis, but is maintained in the custody of another section (e.g. when samples are suspected of containing prohibited substances), this is documented in the UR notes.

Where the dual analysis involves hazardous chemicals or other substances (i.e. drugs, explosives etc) the relevant forensic section is responsible for making a hazard assessment and documenting this in the UR notes for that case. This assessment must include personal risk to staff during examination, storage and subsequent analysis as well as potential risks to equipment.

# 4.7 Managing Worklists

Evidence Recovery is responsible for managing four generic worklists: SAWL, 1BT, ESMP and SALIVA. To access these worklists from AUSLAB main menu:

- 1. Press 5, workflow management
- 2. Press 1, workflow menu 1
- Press 1. workflow lists
- 4. Highlight required list, e.g. SAWL, SALIVA, 1BT and press enter

# 4.7.1 SAWL list

SSLU are responsible for adding cases to this list which involve a sexual element to the offence. Management of the SAWL list is a rostered task. The rostered scientist is responsible for reviewing this list on a daily basis and actioning entries as required:

- Responding to UR note entries as necessary
- Compiling case files (where required) and formulating examination strategies for SAIKs (all examination strategies must be documented in the UR notes for that case and reviewed by a scientist competent to examine SAIKs).
- Where items have been delivered for sexual assault cases, the rostered scientist
  will check to see if the Forensic Relationship field has been completed for each
  item. If a Forensic Relationship has not been entered the scientist will enter a UR
  note requesting this information from the QPS, and place the case on the 1WPP list.



 Checking items received for a Yellow case (particularly in tubes) to determine testing requirements (i.e. if semen screening is required). This may require access to the Forensic Register.

### 4.7.2 1BT list

SSLU use the 1BT list to notify the Evidence Recovery team that action is required for a particular case. The specific advice is recorded by SSLU in the UR notes and may include:

- Testing is no longer required for one or more exhibits
- · A change to the priority of a case
- Additional items have been received
- Requests to contact an QPS officer to provide advice
- Testing is to be re-started or commenced on a previously halted exhibit or case

### 4.7.3 SALIVA list

This list contains all samples that require Phadebas supernatant testing. Refer to QIS document 17193 (Phadebas Test for Saliva) for further details.

### 4.7.4 ESMP list

This list contains reference samples which require manual sampling by an ER scientist. It is important to check whether these samples do require processing, i.e. if a duplicate FTA sample has been received.

# 5 Pre-examination preparation

Before commencing the examination of an item all available case details should be reviewed to determine the type of examination and testing which is required. This information may also be used to prioritise examinations. The following items should be reviewed:

- UR, Specimen and Clinical notes
- Medical notes including SAIK paperwork
- QP127 (if available)
- Forensic Relationship
- Exhibit description

Where the above information does not provide sufficient information to determine testing requirements the following additional strategies may be employed:

- Accessing the Forensic Register (HP5 only)
- Contacting the Investigating Officer, SOCO or Scientific Officer either directly or through SSLU via AUSLAB (1WPP list)
- Contacting the QPS DNA Sample Management Unit
- Contacting FMOs or FNEs

All communications must be recorded electronically in AUSLAB using UR Notes.

**Note:** Specific details relating to the examination of sexual cases are outlined in QIS document 32106 (Examination of Sexual Cases)

# 5.1 Case File Documentation

QIS document 17117 (Procedure for Case Management) describes when a paper case file is required to be created and the process for creating a case file.



When making handwritten examination notes, including making sketches and annotating images, the following general principles must be followed:

- Only approved examination forms (located in QIS) can be used for making examination notes. Printed copies of these forms are kept in the Evidence Recovery laboratory.
- All notes must be legible and in pen.
- All sketches and diagrams must be in pen; however areas of interest and staining may be in coloured pencil.
- Errors must be crossed out once (so that the original item is still visible) and initialled and dated.
- Examination notes must be made contemporaneously and in the examination area.
- Registration of samples must be completed as soon as practicable after the examination.

### 5.2 Amended receipts

Amended receipts are used where there is a discrepancy between the original receipt and the actual contents of the exhibit/s. QIS document 26040 (Procedure for Issuing Amended Receipts in the Forensic Sciences Property Point) describes the process for issuing amended receipts.

### 5.3 FERROs (Forensic non-compliance feedback)

A FERRO should be created when a submission for Forensic DNA Analysis does not conform to our standard operating procedures and is not described in an EXH line.

To create a FERRO, From EXH page:

- Press Shift F10 Registration
- Press End to move cursor to test code box
- 3. Enter FERRO in box, press enter
- 4. F7, F4, F4 (automatically returns to EXH page)
- Pg down to forensic non compliance feedback page
- Press Tab to contact person
- 7. Press Shift F2 bulk edit and enter 'dnaer'. Press F4 to save
- 8. Enter barcode(s). Press F4 to save
- Enter reason. Press F4 to save
- 10. Enter comments as per standard phrasing (see SOP 26071). Press F4 to save
- 11. Press F8 to escape from bulk edit
- 12. Press Shift F12 and enter 'FERRO' to add to list

The Senior Scientist is responsible for reviewing and sending FERROs to the QPS.

# 5.4 Exhibit Delivery

The Property Point deliver exhibits to Forensic DNA Analysis typically once each normal work day (usually in the afternoon). Exhibits are tracked to the Exhibit Room Shelves (FBEXS696-700) or DNA Freezer Shelves (FBDFS1-4). Exhibits may also be stored in other locations within the Exhibit Room or Freezer and must be tracked to their physical shelf location in AUSLAB.

### 6 Examination

Exhibits must be retrieved from their storage location and tracked to the Evidence Recovery laboratory (DAER1).

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# 6.1 Print receipt and labels

The receipt must be printed and attached to the examination notes. To view receipt- press INSERT on the correct casefile page

# To print receipts:

- From the receipt page SF11
- 2. F7 Direct to
- Enter printer name

# The following labels are printed:

- Receipt label to attach to each examination page
- Exhibit barcode (FBLAB6) for examination notes and labelling exhibit as necessary
- Exhibit barcode (FSAMP) to label sample submission tubes

### To print labels:

- From AUSLAB main menu press 7, 1, 3
- Type in label type wanted e.g. FBLAB6 or FSAMP
- 3. Change 'Printer Name' as required e.g. fblabel2
- Press F6. F5
- Scan barcode/s required
- Press Esc from edit mode
- 7. Press F7 to print

# 6.2 Description of packaging

Using QIS documents 17033 (General Examination Record (Unruled)), 17034 (General Examination Record (Ruled)) or 22870 (Forensic DNA Analysis Outer Packaging Record) describe the packing of the exhibit, working from the outer packaging to the inner packaging.

The following minimum details must be recorded for each layer of packaging:

- Packaging type (e.g. HSPB, CSPB, BPB)
- · Seal type, whether the seals are intact and if they are signed and/or dated.
- A brief description of the labelling including unique identifiers (typically barcode).
- QPS outer packaging and all other packaging should be photographed.
- HSPB outer packaging which has been created by Property Point (e.g. for SAIKs)
  does not need to be photographed.

Packaging should be opened in such a way as to maintain the original seals. Where packaging has been opened by it must be signed and dated.

# 6.3 Digital Imaging

Images must be taken for exhibits which are complex and/or difficult to accurately describe in written notes. Smaller, uniform items (i.e. cigarette butts, fingernails, straws etc) do not require images, except where there is unusual staining, damage or other features which are difficult to describe.

A scale and exhibit barcode must be included in every image. QIS document 20080 (Digital Imaging of exhibits in Forensic DNA Analysis) describes the digital imaging process in more detail, including the use of cameras, uploading images to AUSLAB and annotating images.



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### 6.4 Exhibit Numbering

Each exhibit must have a unique barcode registered in AUSLAB, which is used as the common identifier between Forensic DNA Analysis and the QPS. Forensic DNA Analysis assigns a secondary identifier to each exhibit which is a reference to the receipt for that exhibit. Each exhibit is assigned a secondary identifier according to the following format: 123456789-001, where 12345789 is the receipt barcode and each exhibit is assigned an ascending number (i.e. -001, -002, -003 etc).

Subsamples are assigned numbers according to the following example: The first subsample from exhibit 123456789-001 is assigned the secondary identifier 123456789-001-1; the second subsample from the same exhibit is then assigned the secondary identifier 123456789-001-2.

# 6.5 Description of item

Exhibits must be described according to the following minimum requirements:

- What it is
- Size
- Labelling/brand
- Colour
- Staining (including any presumptive tests conducted)
- Physical appearance of damage (without commenting on the cause of the damage)

Staining must be further described using according to its:

- Shape
- Distribution
- Colour
- Size (including measurements)
- Intensity
- Which side of the item the stain may have originated from
- Any presumptive tests performed
- Odour if applicable

Please note that images can be used to describe the physical appearance of stains.

Extraneous surface material such as hairs, glass fragments, fibres and vegetative matter may easily be lost from an item. These materials should be noted in the examination notes, collected into CSPBs (labelled with item barcode) and kept with the repackaged exhibit.

### 6.6 Presumptive or Screening tests

Forensic DNA Analysis uses the following screening tests:

- TMB test for blood see QIS document 17190 (Tetramethylbenzidine Screening Test for Blood)
- AP test for seminal fluid see QIS document 17186 (The Acid Phosphatase Screening Test for Seminal Stains)
- Phadebas test for saliva (paper and supernatant) see QIS document 17193 (Phadebas Test For Saliva)
- P30 test for seminal fluid see QIS document 17185 (Detection of Azoospermic Semen in Casework Samples)
- Microscopy for spermatozoa see QIS document 17189 (Examination For & Of Spermatozoa)



The results of all presumptive testing, including positive and negative control results, must be recorded in the examination notes. Areas on the exhibit which test positive for a presumptive and/or screening test can be circled using a chinagraph pencil or felt tip pen.

Where an examining scientist elects not to perform a presumptive or screening test a justification must be recorded in the Specimen Notes for that exhibit (e.g. that presumptive testing would consume the sample). Where an examination strategy has not been prepared, the examining scientist is responsible for assessing the exhibit and selecting the appropriate presumptive and/or screening tests as per Pre-examination preparation above.

# 6.6.1 Phadebas supernatant testing

Phadebas supernatant testing can be performed following submission of sample for DNA Analysis. This possible screening strategy should be taken into consideration when deciding whether Phadebas screening prior to DNA Analysis will consume the evidence.

Items which are legitimately expected to contain saliva and the presence of saliva is not probative (i.e. cigarette butts, straws, drinking containers) do not require Phadebas testing.

When registering a sample for supernatant testing, the sample must be registered with the Specimen Type 'CSUP' and with "retain s'natant" in the 9PLEX/XPLEX processing comment, and inserted to the SALIVA list.

# 6.7 Sample Selection

The case history, presumptive/screening test results and the staining present on the item are all used to determine which samples, and how many samples are to be submitted. The following elements should be considered when selecting samples for submission:

- Case history offence type and the modus operandi
- Number of offenders if there are multiple offenders/complainants then an increased number of samples may be required to identify as many involved persons as possible.
- Presumptive/screening test results samples of each biological fluid type should be considered for submission.
- Size, location and distribution of staining

# 6.8 Sampling techniques

Forensic DNA Analysis uses the following sampling techniques:

- Swabbing
- Tape-lifting
- Scraping
- Excision
- Submission of whole item

Samples may be either processed directly on a cleaned examination bench, on brown paper and / or within a vessel such as a petri dish (large and small sizes available). The choice of examination surface or receptacle will be determined by the item to be examined and the nature of the examination required

# 6.8.1 Swabbing – used for non-porous surfaces

Swabs are moistened using nanopure water or 70% v/v Ethanol, used to sample the stain, and the entire swab head is submitted for analysis. In some cases a dry swab may be used following the wet swab and both swabs combined in one tube. This strategy should only be used in consultation with the Senior Scientist.

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# 6.8.2 Tape-lifting – used for porous surfaces

The sticky surface of commercial tape is pressed against the item until the tape's adhesive properties are exhausted. Always ensure that a newly exposed section of the tape is used to reduce the chance of contamination. The tape must be rolled with adhesive side in the middle and then placed into a tube for analysis.

### 6.8.3 Scraping

This method is used for fabrics or surfaces where tape-lifting or swabbing are not appropriate and the stain is too large to excise. A scalpel blade (in holder), is used to scrape the top layer of the exhibit, from which a suspension is made or which is submitted for analysis directly.

### 6.8.4 Excision

This method is used for stains/samples which are small enough to fit into a sample submission tube.

# 6.8.5 Submission of whole item

This method is used where the entire item is small enough to fit into a 1.5 or 2ml eppendorf tube.

# 6.9 Specific examination strategies

# 6.9.1 Examination of clothing / footwear for epithelial cells

Generally only a small number of epithelial cells are deposited by touching or wearing items. It is best to use one side of a swab or a piece of tape no more than 2cm long to collect for submission, so as to concentrate cellular material into one sample.

High friction areas, including armpits, collars, inside collarbone, waist bands, hat bands and other parts of clothing that are in constant contact with the wearer are good areas to sample.

# 6.9.2 Syringes

Specific Syringe Handling Kits are available to make this process as safe as possible. Refer to QIS document 17135 (Handling and Sampling of Syringes and Needles).

### 6.9.3 Swabs

Record the amount of the swab that is stained, the colour, the stain intensity, the result of any screening tests and the amount of the swab that is submitted for DNA analysis. The entire swab head can be cut off and submitted for testing. A specific form is available for recording: QIS document 22846 (General Swab Exam Record).

# 6.9.4 Cigarette Butts

When examining cigarette butts, indicate whether or not the cigarette appears to have been smoked, whether it has been stubbed/flattened and any brand names visible on the butt. A specific form is available for recording: QIS document 23014 (Cigarette Butt General Examination Record). When sampling cigarette butts, any tobacco and/or filters are removed during sampling and not submitted for testing.



Smoked manufactured cigarettes: Excise a 0.5cm circumference of the filter paper from the butt using a scalpel blade and submit for testing.

Smoked hand rolled cigarettes: Submit entire cigarette paper for testing.

Unsmoked manufactured and hand-rolled cigarettes: Submit the entire cigarette paper and filter paper for testing. If there is too much substrate for one tube, the sample must be submitted for extraction in multiple tubes and then pooled post extraction (see Appendix 2: Pooling).

For all cigarette butts, once sampling has been completed, any remaining portion of the item (excluding filters and tobacco) are retained in the item retention box, for further testing if required. If the examining scientist believes that a substance other than tobacco is contained with cigarette, this must also be retained in the item retention box.

Where a CSSE contains multiple cigarette butts, and not all cigarette butts have been submitted for analysis, the Sample Info 1 fields are used to communicate to the QPS the total number of cigarette butts contained in the CSSE and the number of cigarettes which have been tested. To do this, complete the Sample Info 1 fields as per below:

- For the Parent exhibit add to the end of the existing item description add 'N total cig butts' where N is the total number of cigarette butts in the CSSE.
- For each cigarette butt submitted for tests add 'cig butt 1 of N', 'cig butt 2 of N' etc.

### 6.9.5 Fingernails

Fingernails or fingernail scrapings are examined to find blood or cells on or under the nails. Clippings, loose scrapings, scrapings on swab sticks or complete nails may be submitted. Describe the fingernails in terms of number, size and any visible staining. Submit these items for analysis (noting if all or some submitted). Generally samples for each hand are pooled, i.e. all samples from the left hand together and all samples from the right hand together.

Samples are not pooled where the case circumstances require:

- seeing which finger was used in digital penetration
- where the items are whole swabs
- where there are TMB positive and TMB negative samples.

# 6.9.6 Post Mortem samples

The examining scientist is responsible for assigning an EXH barcode to the PM samples as a whole, which must be communicated to the Senior Scientist who will forward this information to DRMU via QPS email. The receipt under which the samples are submitted usually has an associated Coronial case number. Before any subsamples are registered this Coronial case number needs to be changed to the associated QP number by an AUSLAB Corrections Officer. If subsamples are registered under a Coronial case number the EXH lines will not be transmitted to QPS.

PM samples may include sexual assault swabs and/or slides (high vaginal, low vaginal, vulval etc), body swabs, pubic hair, head hair, fingernail clippings or scrapings.

Intimate swabs (i.e. those taken for a sexual offence including vaginal, anal and oral) are examined according to standard SAIK examination procedures. However, semen negative intimate PM swabs are all submitted for analysis - refer to QIS document 32106 (Examination of Sexual Cases).

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Sometimes the fingernail clippings include a portion of tissue or part of the finger. In this case a moistened swab can be used to sample potential foreign DNA from the underside of the nail, taking care not to sample the deceased person's tissue (i.e. targeting the distal end of the nail).

# 6.9.7 Heavily stained items and known infectious items (universal precautions)

Heavily stained or soiled items, or items containing known infectious material may be examined in the fume hood to prevent contamination and infection. Double gloving should be considered during examination, as this provides an additional physical barrier between the examiner and the item.

# 7 Sample registration

# 7.1 CS page

The case status must be updated:

- If the status is 'Started' no change is required
- If the status is 'Received' it must be changed to "Allocated' then to 'Started'
- If the status is "Allocated" then change to "Started"
- If the status is 'Report Issued', or 'Analysed- Report not Required', change the status to 'Reactivated'
- If the status is 'Reactivated', 'Sent to Peer Review' or 'Return from Peer Review', no change is required

Ensure that the Team is entered on the CS page and also in the BTEAMS field on the registration (SF10) page. The Priority listed on the CS page should be used as the priority for all samples submitted for that case (unless otherwise specified in the UR notes).

# 7.2 EXH entry

An appropriate EXH line (or multiple lines) must be entered on the EXH page for each exhibit. QIS document 23008 (Explanations of EXR/EXH Results) provides a list of all EXHs and their expanded wording.

Enter the appropriate barcode for the EXH line, which may be the exhibit barcode (where the result refers to the entire item, or the entire item is submitted for testing), or a subsample barcode. There is no need to enter duplicate EXH lines where multiple subsamples are submitted for the same analysis.

To enter an EXH line:

- Press 3 Patient Enquiry
- Scan exhibit barcode
- Press Shift F2 to edit the page
- 4. Fill in appropriate EXH lines with barcodes and results/status (Use F1 look-up)
- 5. Fill in team name (if not already populated)
- 6. Press F8 to exit edit mode

# 7.3 Registration of exhibits

Sample registration, from EXH page:

- 1. Press Shift F10 Registration and complete the following fields:
- 2. Specimen type e.g. EXHIBITS (if there are sub-samples) or FSS
- 3. Primary site e.g. SWAB, CIG BUTT
- Client Ref (receipt # 00x)
- DNA priority



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- Sample Info 1 description of the sample, any relevant barcodes and micro result if applicable
- 7. Enter 'FBX' in box, press enter and follow prompts at bottom of page
- 8. Enter '9PLEX/XPLEX' in box, press enter (for swabs, cig butts and items consumed by sampling) A prompt will appear enter in any necessary processing comments, e.g. ~150ul nano H20 added.
- 9. Press F7, F4, F4 (automatically returns to EXH page)
- 10. SHIFT F9 to view summary page

Note: If the item is a piece of clothing, item ownership can greatly assist with DNA profile interpretation. If there is no indication within the item details / descriptions transferred from the Forensic Register or on the item packaging as to whom the item of clothing is attributed to (or if is unknown), including information within the Forensic Relationship field (or if this information is not informative e.g. when there is more than one victim or suspect), list insert the exhibit barcode onto the Item Ownership List "ELF" for SSLU to follow-up with QPS. If there are no reference samples associated with the case at the time of examination, this is not necessary.

Commonly used specimen types include:

- HAIR or HDNA: for samples that have been identified as human hair suitable for DNA analysis.
- SFRAC/EFRAC: for samples that have semen present.
- CSUP: for samples that are to have their supernatants retained for phadebas amylase activity testing. Enter processing comment "retain s'natant". Sample should then be placed on SALIVA communication list.
- NUCT: for tissue samples

Repeat procedure for other exhibits/ samples if required.

# 7.4 Registration of sub-samples

Sub-sample Registration

- From EXH page:
- 2. Press Shift F10 Registration
- 3. Press F7, F4, Shift F5 to copy page
- 4. Scan in new barcode for sub-sample and complete the following fields:
- 5. Specimen type e.g. FSS
- 6. Primary site e.g. SWAB
- 7. Client Ref (receipt # 00x-y)
- DNA priority
- 9. Sample Info 1 description of the sample, barcode of exhibit the sub-sample is taken from
- 10. Enter '9PLEX/XPLEX' in box, press enter. A prompt will appear enter in any necessary processing comments e.g. :retain s'natant"
- 11. F7, F4, F4 (automatically returns to EXH page)
- 12. SHIFT F9 to view sub-samples on summary page
- 13. Enter on sub-sample and add 'Team' name if not already present

Repeat for other sub-samples if necessary.

# 7.5 Consumables and reagents

For each tube that is used, details of the tube lot number must be recorded in the audit trail for that sample barcode. Reagent details must be attached to all samples where a reagent





has been used (e.g. when TMB screening performed) with an EXH page – in the case of an item with multiple sub-samples the reagents only need to be logged against the item EXH.

- 1. From the EXH page or from the 9PLEX/XPLEX page for sub-samples
- Press F12
- Press F5 consumables
- Press F5 Add Entry
- 5. Use F1 look-up list and arrows to highlight required consumable or reagent
- Press Enter
- 7. Press F6 Add Notes to record bag number or details of box number e.g. P30 kits
- Press F4 to save
- Press ESC only when you are ready to escape and the reagent has been logged.

To see that the items have been assigned, from the EXH screen, press F12, F5

### 7.6 Sample tracking

When registration is complete samples must be tracked to an ERT transfer box and placed in the transfer hatch. Transfer boxes not collected and transferred to Analytical must be stored at close of business to the Exhibit Room (FBEXS700) or if the samples are wet to the Freezer (FBDFS1).

To Store sample tubes:

- 1. From main menu
- 2. Press 2 sample processing
- Press 6 sample storage
- Scan the barcode of the storage box
- 5. Press Shift F5 to fill the rack
- Scan the barcode on the tube and place tube in rack position as indicated on the screen.

# 7.7 Exhibit repackaging and return

Exhibits should be repackaged in the same packaging if practical. Re-seal the openings with evidence tape, sticky tape or heat seal and initial and date the seal.

If an exhibit is wet as the result of examination, it can be placed on the drying rails overnight to allow it to dry. Ensure that the rails are cleaned with bleach and ethanol before and after drying. Exhibits must have a piece of brown paper between the rail and the item, and an additional piece of brown paper covering the item.

If examination of an exhibit is not complete, the item can be tracked back to the freezer or the exhibit room and retrieved at a later date. Where the examination is complete the exhibit must be tracked to the Exhibit Room return location (FBEXR1) or the Freezer returns location (FBEFR1).

To transfer an Exhibit, from the EXH page:

- 1. Press Shift F9 summary page
- Press Shift F5 to show storage locations
- Highlight line for receipt/exhibit barcode of interest
- Press Shift F7 to transfer item
- 5. Enter storage location where you want to move it to and press Enter
- 6. You will be prompted to confirm transfer. Press Y. and Enter
- Check item of interest has been transferred to desired location



# 7.8 Tracking case files and examination notes for review

After examinations and registrations are complete, all notes are to be tracked to the DAPR1 drawer to be peer reviewed by another competent scientist and the EXH lines reviewed and validated. Refer to QIS document 17117 (Procedure for Case Management) for the review of EXH lines.

Once examination notes have been reviewed they are to be filed in one of the following locations:

- If the case is paperless, track the receipt to FBSI47 for filing by the admin team.
- If you are in possession of the case file, add the notes to the file and track to the back section of the DAPR1 drawer. Case files will stay in this location until they are transferred once weekly to a case management drawer.
- If the case file is stored to another location and you have an additional receipt, add
  the notes to the case file and remove the location of the receipt in AUSLAB, and
  track the receipt to the Case Management drawer (FBCM24).

# 8 Examination and sampling of reference samples

All samples that are designated as reference samples **must** be examined and sampled on **Examination Bench 15**, and this bench is used solely for this purpose. Reference samples for processing are added to the ESMP list and stored to the ESMP box located on Exhibit room shelf 702. Once completed, items are stored to FTAbox 660 located on the same shelf.

# 8.1 Examination of reference samples other than FTA cards

The principles of examination and sampling of reference samples other than FTA cards (e.g. swabs, hairs) are the same as those for casework exhibits. Refer to Examination above. The specific registration of reference samples is different refer to Appendix 3: Reference sample registration for the registration of reference samples.

### 8.2 Examination of FTA reference samples

For reference FTA cards, determine if the card is being sampled due to either:

- a) the unavailability of the BSD instrument for extraction preparation or if the process is being performed in Evidence Recovery due the urgent nature of a case. Or,
- b) due to failure to gain sufficient DNA from routine extraction preparation processes, and additional sample is required to yield a DNA profile.

Note: All blood FTA cards are to be sampled by ERT and not processed through the BSD.

8.2.1 For FTA cards being sampled for routine extraction preparation when BSD instrument is unavailable.

For these samples, an EREF test code should be visible in the SF7 Results History table. There should be no results against the EREF test code, the specimen notes should state something similar to "to be sampled by Evidence Recovery". Additionally, within the sample audit trail, the EREF test code should be outstanding on an "R21EXT" batch. If the sample has been extracted previously, a connected barcode may be registered, specimen notes will indicate under which barcode the FTA card is to be sampled. If it is unclear which barcode is to be used, or if a connected barcode is required, do not proceed with sampling and consult the Senior Scientist

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- 1. Excise an approximately 5 mm x 5 mm section from each of the black circles on the FTA card and place into an appropriately labelled 2 mL screw capped tube.
- Register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.

**Note:** If the FBEXAM and specimen note are to be recorded against the barcode that the sample is being processed under. This may be a connected barcode.

- 3. Add details of tube lot number (refer section Consumables and reagents above
- Notify the Quality and Projects senior scientist, the Operational Officer supervisor or the Analytical Team senior scientist that sampling is complete and that the sample is ready to for DNA extraction.
- 5. Add a specimen note stating that sampling is complete.
- 8.2.2 Blood FTA cards being sampled for the first time
  - 1. Excise an approximately 5 mm x 5 mm section from each of the black circles on the FTA card and place into an appropriately labelled 2 mL screw capped tube.
  - 2. Register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.

**Note:** If the FBEXAM and specimen note are to be recorded against the barcode that the sample is being processed under. This may be a connected barcode.

- Access the SF7 results history table, order an EREF test code using the SF8 add rework function.
- 4. Add a specimen note stating that sampling is complete.
- 8.2.3 For FTA cards being sampled due to insufficient DNA from previous extraction processing.

These samples will be being processed as a final attempt to gain a DNA profile after routine methods have failed. As such, as much remaining sample as is reasonable is to be sampled for profiling.

- Determine whether a connected barcode has been registered for this sampling process. For the registration of connected barcodes for reference samples refer to QIS document 17117 (Procedure for Case Management).
- Excise as much sample as is feasible and place in an appropriately labelled 2 mL screw capped tube.



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- 3. Under the connected barcode, register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.
- Notify the Quality and Projects senior scientist, the Operational Officer supervisor or the Analytical Team senior scientist that sampling is complete and that the sample is ready to for DNA extraction.
- 5. Add a specimen note stating that sampling is complete.
- 8.2.4 Blood Cloths that have not been previously registered in AUSLAB
  - 1. Request registration [1]
  - Full Reception Entry [1] Fields to fill in are:

Lab Number - Scan new assigned barcode #

UR/Case No. – Add the P number from the blood cloth ((this may auto populate some fields depending if it has anything registered on the number previously) Surname. Given Names – if known

Loc./Client – QPS (Queensland Polices Services)

Collected - the current date

Received - the current date

Specimen - RCELLS (reference cells)

Primary Site - MISC

DNA Priority – should be requested from information given (usually a 2)

Sample Info 1 - Add the name

Test codes – REF21 & FBX

If page did not self populate with information, it will need to be linked to the parent case:

- [F6] Associated Crisps
- [F5] Add Crisp
- Enter QP number (should be in email request for blood cloths)
- Enter QP number again

[F7] Billing

Add current date to Requested field.

[F4] [F4] to exit

- 3. Enter back into new lab#
- 4. Enter into registration [Shft-F10
- 5. Change Specimen to RBLOOD (Reference Blood)
- 6. Change Primary Site to BLCTH (blood cloth)
- 7. Save and exit [F7] [F4] [F4]
- 8. Add a specimen note stating that sampling is complete.



### 9 Associated Documentation

QIS: 16004 - AUSLAB Users Manual - Forensic DNA Analysis

QIS: 17033 - General Examination Record (Unruled)

QIS: 17034 - General Examination Record (Ruled)

QIS: 17117 - Procedure for Case Management

QIS: 17119 - Procedure for Release of Results

QIS: 17135 - Handling and Sampling of Syringes and Needles

QIS: 17140 - Procedure for the Identification and Examination of Hairs

QIS: 17185 - Detection of Azoospermic Semen in Casework Samples

QIS: 17186 - The acid Phosphatase Screening Test for Seminal Stains

QIS: 17189 - Examination For & Of Spermatozoa

QIS: 17190 - Tetramethylbenzidine Screening Test for Blood

QIS: 17193 - Phadebas Test for Saliva

QIS: 20080 - Digital Imaging in DNA Analysis

QIS: 22846 - General Swab Exam Record

QIS: 22857 - Anti-contamination Procedure

QIS: 22870 - Forensic DNA Analysis Outer Packaging Record

QIS: 23008 - Explanations of EXR/EXHs

QIS: 23014 - Cigarette Butt General Examination Record

QIS: 23055 - General Examination Record

QIS: 23849 - Common Forensic DNA Analysis Terms and Acronyms

QIS: 23898 - SAIK Details Record

QIS: 26071 - Examination of In-tube samples

QIS: 31286 - SAIK form no semen testing

QIS: 32106 - Examination of Sexual Cases

QIS: 32639 - General Examination Form (Packaging) with microscopy

QIS: 32640 - General Examination Form with microscopy

# 10 Amendment History

Revision	Date	Author/s	Amendments
0	17 Feb		
	1999		
1	26 Jun	V lentile	
	2001		
2	18 Sep	V lentile	Amendments to references, (8)
	2002		Characterisation of Biological
			material and (22) Reference

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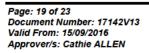


			Samples
3	26 May	K Lee	Entire document revised and
3	2005	IN Lee	rewritten
4	21 Oct 2005	M Candana	
4	21 Oct 2005	M Gardam	Added when to use "what appears
			to be" for when a confirmatory test is
			not available.
5	22 June	M Gardam	Added Techniques for various
	2006		exhibits, added AUSLAB
			Flowcharts.
6	14 Feb	L Weston	Update with new processes for
	2007		AUSLAB-LIMS
7	14 Jan	J. Connell	Added: size references for DNA
<b>'</b>	2009	J. Munoz	extractions; destruction
	2003	3. Wanoz	
			requirements; dual examinations;
			swab and tape lift brands used;
			associated documents; table of
			contents. Removed appendix &
			reference to volume flowcharts.
			Updated processes for: off deck
			lysis; registration of multiple items.
			Separated General Examination
			Procedure into subheadings.
			Transferred information for case file
			compilation and AUSLAB
			procedures from Case Management
			SOP. Transferred into new template.
			Changed EXRs to EXR/EXHs.
			I CHAHUEU EARS 10 EAR/EARS.
OIC2 Editio	n		
QIS <sup>2</sup> Editio		Undated By	-
Version	Date	Updated By	Amendments
	Date 25 August	Updated By K.Scott	Amendments Updated requirements as per NATA
Version	Date		Amendments Updated requirements as per NATA assessment DEC 2008.
Version	Date 25 August		Amendments Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails.
Version	Date 25 August		Amendments Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration
Version	Date 25 August		Amendments Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6
Version	Date 25 August		Amendments Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including
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Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6  Add submission of wet/dry swabs from skin or other items.
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6  Add submission of wet/dry swabs from skin or other items.  Add submission of TMB pos. TMB
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6  Add submission of wet/dry swabs from skin or other items.  Add submission of TMB pos. TMB neg fingernail scrapings  Add N=No further work in Foren.Rel.
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6  Add submission of wet/dry swabs from skin or other items.  Add submission of TMB pos. TMB neg fingernail scrapings  Add N=No further work in Foren.Rel. Field.
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008.  Updated FIRMU contact emails.  Addition of examination/registration of a reference sample – including appendix 6  Add submission of wet/dry swabs from skin or other items.  Add submission of TMB pos. TMB neg fingernail scrapings  Add N=No further work in Foren.Rel. Field.  Add references to SOP 26071
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements
Version	Date 25 August		Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms
Version 9	Date 25 August 2009	K.Scott	Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms section
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Version 9	Date 25 August 2009  October 2013  6th December	L. Ryan A Houlding J Seymour-Murray	Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms section  Split Examination of Items SOP in sexual and non-sexual SOPs. Rework entire SOP content  Changed DNA Analysis to Forensic DNA Analysis, added XPLEX,
Version 9	October 2013  6th December 2013	L. Ryan A Houlding J Seymour-Murray  J Seymour-Murray	Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms section  Split Examination of Items SOP in sexual and non-sexual SOPs. Rework entire SOP content  Changed DNA Analysis to Forensic DNA Analysis, added XPLEX, updated Appendix 3.
Version 9	Date 25 August 2009  October 2013  6th December	L. Ryan A Houlding J Seymour-Murray	Amendments  Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms section  Split Examination of Items SOP in sexual and non-sexual SOPs. Rework entire SOP content  Changed DNA Analysis to Forensic DNA Analysis, added XPLEX,

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	2015		samples, updated to new template. New multiple items screen shot. Combined 11.3.4 & 11.3.5 into 11.3.3
13	24 August 2016	A McNevin	Updated section 8 processing of Reference samples and minor updates to sections 6.8 & 4.1, small formatting changes





# 11 Appendices

# 11.1 Appendix 1: Registration of multiple items

Where QPS submit multiple items under the one barcode, each item can be individually assigned a barcode and an EXH ordered, so that each item can be reported separately to the QPS. The screen shot below shows a parent item which contains three items (white shirt, red shorts and black hat). The EXH has been entered so that each of these three items can be registered under the Linked No. barcode, and an EXH ordered for each of these items.



Figure 1: EXR/EXH page for original barcode

### 11.2 Appendix 2: Pooling

The example below is based on the following examination scenario:

One parent item, an unsmoked cigarette butt (lab# PARENT), is to be sampled into two subsamples: Subsample A (lab# SSA) and Subsample B (lab# SSB). These two subsamples will then be pooled into one sample: Pooled (lab# POOLED).

PARENT barcode is the existing EXH barcode which has been transferred from the Forensic Register. SSA, SSB and POOLED are all new barcodes which must be registered by the examining scientist using standard item registration methods.

Actions for Parent Item – Unsmoked Cigarette Butt (lab# PARENT) Registration:

Client ref: -001

Test Codes: EXH, FBEXAM

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Leave as transferred from Forensic Register

### EXH:

 EXH line: submitted results pending, using lab# POOLED as this will be the reported lab#

Actions for Subsample A (lab# SSA)

### Registration:

Client ref: -001-1

Test Codes: 9PLEX/XPLEX, POOLED

Processing Comment: Ext & Hold

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Parent Item description; Sub Sample A

9PLEX Page only (these fields are not relevant for XPLEX pages):

Accepted Barcode: #POOLED

Connected Barcodes: #SSB, #POOLED

# Pooled Page:

- This lab number has been pooled with Lab Number: #SSB
- Processed Using Lab Number: #POOLED
- Reported Under Lab Number: #POOLED

Actions for Subsample B (lab# SSB)

### Registration:

Client ref: -001-2

Test Codes: 9PLEX/XPLEX, POOLED

Processing Comment: Ext & Hold

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Parent Item description; Sub Sample B

9PLEX Page only (these fields are not relevant for XPLEX pages):

Accepted Barcode: #POOLED



Connected Barcode: #SSA, #POOLED

# Pooled Page:

- This lab number has been pooled with Lab Number: #SSA
- Processed Using Lab Number: #POOLED
- Reported Under Lab Number: #POOLED

# Actions for Pooled (Lab# POOLED)

# Registration:

- Client ref: -001-3
- Test Codes: 9PLEX/XPLEX
- Processing Comment: nil
- Specimen Type: POOLED
- Primary Site: leave blank
- · Sample Info 1: SSA, SSB, Parent Item description in Sample Info 1

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# 11.3 Appendix 3: Reference sample registration

# 11.3.1 Registration of reference tissue from a coronial

Take the following steps to register the sample:

- Locate the receipt page for the coronial samples
- 2. Access the registration page for the receipt (Shift F10)
- Copy the page (F7, F4, Shift F5)
- 4. Scan the new barcode for the reference tissue
- Specimen Type: Nucleospin-Cells-Ref (NUCCR)
- Primary Site: leave blank
- 7. Client Ref: receipt barcode
- 8. Sample Info 1: enter a description of the tissue (e.g. liver)
- 9. Test Codes: FBEXAM, REF21
- 10. Save the registration (F7, F4, F4)

# 11.3.2 For Blood Swab/ Blood Cloth

Specimen Type: Reference Blood

Test Code: REF21

# 11.3.3 For Cell Swab/ Reference Hair (for samples in ESMP box)

- 1. Note down the delivery person and receiving person details
- 2. Enter into the registration page (SF10)
- Add FTAR test code
- Delete REF21 & FTAREF test codes
- Change specimen type to RCELLS/RHAIR
- 6. Save registration (F7, F4, F4)
- Re-enter the SF10 registration page
- Delete FTAR test code
- 9. Add FTAREF & REF21 test codes
- 10. F7,F4,F4 to save
- Check audit trail, it should be on a RFIQMAX batch.





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Forensic DN/ MICROSCOPY		Item Barcode:		Page: Date: Time:		
Description	Whole Sperm	Sperm Heads	Epithelial Cells	Comments (e.g. Bacteria; cellular or non-cellular debris Red Blood Cells; White Blood Cells; Yeast Fungi; Fibres)		
Grading system:  0 = None seen <+ (<1) = Very hard to find (<10 use England Finder) + (1+) = Hard to find ++ (2+) = Easy to find +++ (3+) = Very easy to find ++++ (4+) = Abundant  COMMENTS:			<b>—</b> 4			

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# Forensic and Scientific Services

Sexual Assault Inve						
Forensic DNA Analysis, Forensic and So				Forensic and S	cientific Services, Department of Health	
SURNAME:					GIVEN NAMES:	
DOB:					SEX: Male  Female	
Examining Do	octor / FMO / FN	NE:			Investigating Officer:	
Address of ex	aminer:				Police Station:	
E-mail address:					E-mail address:	
Contact Phon	e Number				Contact Phone Number:	
Date of SAIK					Date of Assault:	
Time of SAIK	Examination:				Time of Assault:	
		lete for worksp number or loca		ent Yes	s No 🗆	
TYPE OF	Digital	Penile	Oral	Object		
ASSAULT:		penetration		penetration	PREVIOUS SEXUAL ACTIVITY: Yes ☐ >7 days previous ☐ Date (if ≤ 7days) :	
Oral					No □	
Vaginal					Was previous coitus with suspect? Yes ☐ No ☐ Unknown ☐	
Anal					Was condom used with previous coitus? Yes ☐ No ☐ Unknown ☐	
Penile					For Female SAIK	
Other	(please provid	de details in "Su	ımmary of Assa	ult"):	Date of last LMP:	
ASSAULT DE	TAILS				CASE HISTORY	
Was a condo	m used:	Yes 🗌 No 🗀	Unknown		Has the patient consumed a substance which may alter consciousness?	
Did ejaculatio	n occur:	Yes 🗌 No 🗀	Unknown 🗌		Yes ☐ No ☐ Unknown ☐	
Was a lubrica	nt used:	Yes 🗌 No 🗆	Unknown		Can the patient recall and communicate the incident?	
(if yes, please	provide details	in summary of	assault details	section)	Yes □ No □ Unknown □	
					Did the assault involve any kissing / licking / biting?	
How many offenders were involved: Male Female					Yes ☐ No ☐ Unknown ☐	
		nown, please d	etail within sum	mary of	If yes, locations:	
assault details section)					Was there any genital / anal injury causing bleeding?	
OINCE THE		THE DATIEN	<b>.</b>		Yes □ No □	
	•	STHE PATIENT		o □ No□	If yes, details:	
_	thing: Yes 🗌 I ning: Yes 🗍 I		Douched: Ye Urinated: Ye	s □ No □		
	athed:Yes □		Defecated: Ye			
Washed Mou	<del>-</del>	_	Vomited: Ye			
Cleaned Teet			drink / food: Ye			
SUMMARY C			e provide as mu	ch information	as possible as this assists with DNA Analysis):	
					(continue on next page)	

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Sexual Assault Investigation Kit (SAIK) Forensic DNA Analysis, Forensic and Scientific Services, Department of Health						
SUMMARY OF ASSAULT DETAILS	(continued from previous):	_				
			_			
			_			
			_			
CLOTHING AND OTHER ITEMS						
Item Clothing / Sanitary Items: Details:						
Worn pre-assault:	Worn post-assault □	Worn pre- and post-assault □				
Item Clothing / Sanitary Items: Details:						
Worn pre-assault: □	Worn post-assault □	Worn pre- and post-assault ☐				
REFERENCE SPECIMENS: (please p	backage in a separate envelope within the	SAIK)				
FTA Sample (preferred)	Yes  No Buccal Cells	Blood				
Blood reference sample	Yes \( \square\) No \( \square\) 5ml EDTA tube					
Oral reference sample (May only be used by Forensic DNA A	Yes ☐ No ☐ Analysis if no allegation of oral assault or n	o history of loss of consciousness)				
Blood sample taken for Toxicology?	Yes No 🗆	Urine sample taken for Toxicology?	Yes No 🗆			

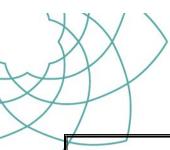
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Sexual Assault Investigation Kit (SAIK) Forensic DNA Analysis, Forensic and Scientific Services, Department of Health							
Please o	Please document the samples collected for DNA below						
FEMALE	FEMALE SAIK SWABS:						
	High vaginal			☐ Low vaginal			
	Vulval			Cervical			
	Perianal			Rectal			
	Oral			Other			
	Other			Other			
MALE S	AIK SWABS:						
	Base of penis			Shaft of penis			
	Glans penis			Scrotum			
	Perianal			Rectal			
	Oral			Other			
☐ Other			Other				
BODY/SKIN SWABS: (please specify if biting, licking or kissing has occur			ed at site	from which bod	y swab was collected)		
	Location			Location			
	Location			Location			
	Location			Location			
	Location			Location			
Location			Location				
FINGERNAIL CLIPPINGS/SCRAPINGS:					DROP SHEET		
	Left Hand	☐ Right Hand					

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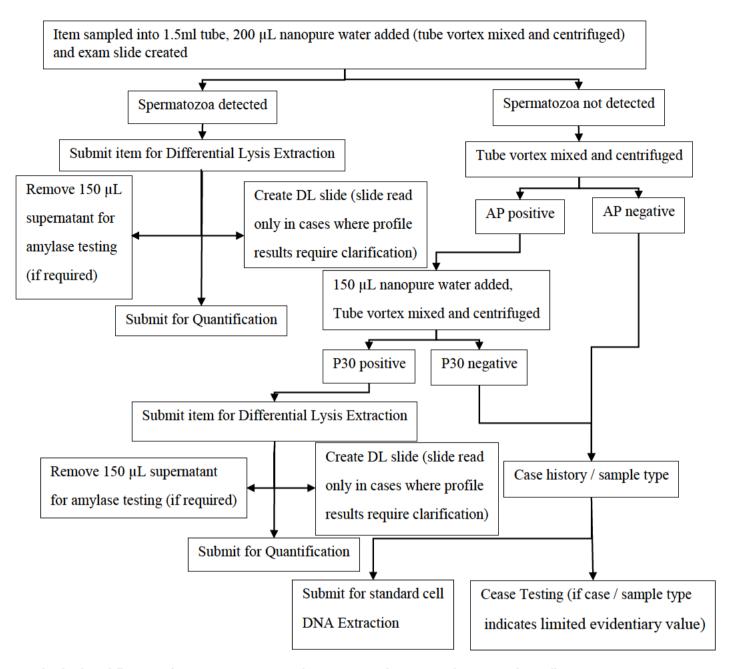
# Health**Support**Queensland

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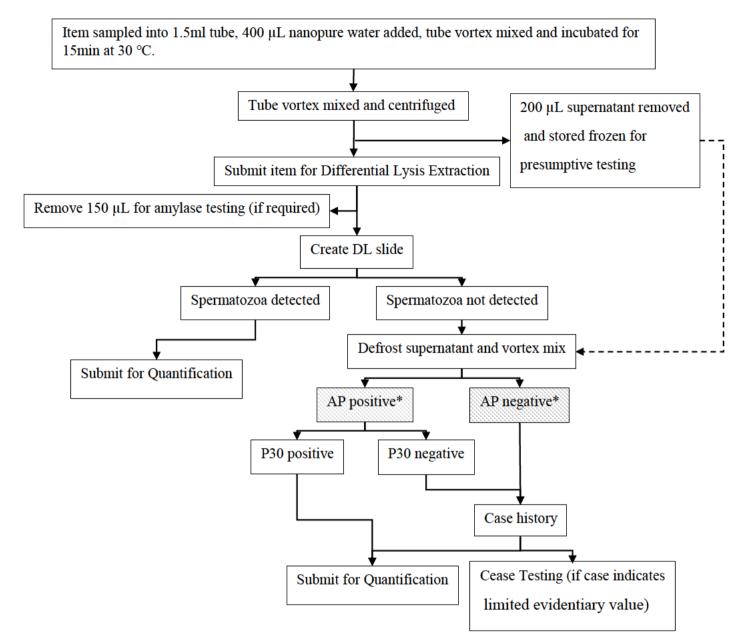
		Case:	Page:
Forensic DNA Analysis SAIK DETAILS RECORD		Receipt No:	Date:
SAIR DETAILS RECORD			Time:
Barcode			
Labelling details  Barcode/unique identifier present on SAIK when received	12 Vas 🗆	No □	
Labelled in part:	. 100 🗀	но 🗀	
See image/s in AUSLAB			
Details of seal			
Original Seal			
Sticky tape   Evidence tape   Glued   Stapled	_ Tamper	evident seal 🗌 Othe	er
Is original seal intact?			
Yes 🗌 No			
Signed Yes ☐ No ☐	Is opening	g signed? Yes 🗌 N	lo 🗌
Dated Yes ☐ No ☐	Is opening	g dated? Yes 🗌 N	lo 🗌
Has the SAIK been opened by Property Point staff (some	where othe	er than original seal)?	•
Yes 🗌 N	o 🗌		
Signed Yes ☐ No ☐			
Dated Yes ☐ No ☐			
Contents of SAIK			
	Examined	Not Examined	Appears unused
Comments:		Exam bench:	Examined by:

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Method 1 (workflow used in Forensic DNA Analysis prior to this project being conducted)



<sup>\*</sup> Due to poor AP performance throughout this study, it was decided upon implementation of Method 2 that standard AP testing would be ceased, and only p30 would be used to screen for seminal fluid

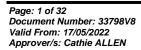
Method 2 (Workflow being testing during this project)

## **Queensland Health**

# Forensic and Scientific Services



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#### 1 Purpose

The purpose of this procedure is to describe the processes required for the examination of sexual assault cases by Evidence Recovery scientists and technicians in Forensic DNA Analysis, in addition to those described in QIS <u>33800</u> Examination of Items.

#### 2 Scope

This procedure applies to all Forensic DNA Analysis staff that examine or interpret examinations of evidentiary items. This standard operating procedure is used in conjunction with individual methods for screening tests. Interpretations and limitations of reporting are to be found in each method.

#### 3 Definitions

- Refer to QIS 23849 (Common Forensic DNA Analysis Terms and Acronyms) for a comprehensive list of abbreviations.
- All references to microscopy, refer to QIS <u>17189</u> (Examination For & Of Spermatozoa).
- All references to Acid Phosphatase (AP), refer to QIS 17186 (The Acid Phosphatase Screening Test for Seminal Stains).
- All references to Phadebas, refer to QIS 33998 (Phadebas Test For Saliva).
- All references to Tetramethylbenzidine, refer to QIS 17190 (Tetramethylbenzidine Screening Test for Blood).
- All references to p30, refer to QIS <u>17185</u> (Detection of Azoospermic Semen in Casework Samples).

#### 4 Actions

Refer to the general principles contained in QIS 33800 Examination of Items.

#### 4.1 Sexual Assault Investigation Kits (SAIKs)

Before commencing the examination of a SAIK an examination strategy must be devised and reviewed in accordance with Section 4.12.1 of this document by different scientists that are competent to perform the examinations contained in the strategy. This strategy must include:

- For each item to be examined, what biological fluid is to be screened for,
- Items which require no further action,
- Sample submission strategies (i.e. extraction type, pooling, retain supernatant for Phadebas testing etc.).

The following are general principles which are used to develop examination strategies for SAIKs, however these principles must be considered within the context of the case history:

- Where the complainant is a minor or has an intellectual impairment, which may mean that the provided case history is unreliable, all possible offence scenarios are considered,
- Where the complainant is an adult who has lost consciousness, has impaired memory or has consumed alcohol or drugs prior to or during the offence which may impact on memory, all possible offence scenarios are considered,
- Consider previous intercourse with same or different partner, prior to the offence.
   For digital only female complainant cases with prior intercourse, submit external swabs for diff lysis with no presumptive testing.



- For male offender SAIK swabs, consider submitting penile swabs for diff lysis where the victim has had previous intercourse with another person,
- Consider the number of offenders for male SAIKs consider submitting penile swabs for diff lysis (with no presumptive testing) to separate epithelial cells and spermatozoa,
- Samples taken from areas of biting, licking or kissing (or other oral contact) are submitted for presumptive saliva testing (retain supernatant). This does not include swabs taken from the mouth (internal or external), anal and vaginal areas which may give false positive results,
- Internal and external vaginal swabs can be submitted for retain supernatant only if the female is under 16 years old,
- For internal swabs on adult females (16 years and older), an Analytical Note for the Epithelial Fraction to be processed as "Extract and Hold on EFRAC" is required. This rule applies for both SAIK swabs and PM SAIK swabs. This does not apply to priority 1 samples.
- If an oral swab has been received which is labelled as a reference, the SAIK is to be
  placed on hold and a request/task sent for further advice from QPS. If QPS advise
  that the sample is not required as a reference it can be examined accordingly with
  the other SAIK contents.

If FMO prepared slides are received within a SAIK, the following workflow applies:

- Where the swab and smear are clearly labelled the same (can be identified as matching), create a microscopic entry under the swab barcode and record the slide labelling details in the notes field. Stain and examine the slide, if sperm is observed the diff slide from the examined swab does not require reading after extraction.
- In the instances where the pre-prepared smear is microscopy negative, proceed with routine processing.
- If the swab and slide cannot be connected (e.g. unlabelled or labelled differently) register the slides as separate child exhibits and perform microscopy. Proceed with routine processing of the swabs.

Additional items such as pads, tampons or fluid samples received within a SAIK are to be examined at the same time as the rest of the SAIK samples, refer to section 4.5 for testing requirements for sanitary items and section 4.20 for examining fluid samples.

If there are any issues relating to the collection or documentation of a SAIK, send a request/task to the forensic officer or to SSLU, refer to QIS 33771 to create and complete a request/task\_and QIS 33800 for standard wording. In some cases the FMO / FNE (direct or through SSLU) may need to be contacted. Examples of issues include:

- Missing paperwork
- Insufficient case history to determine an examination strategy
- Labelling issues/inconsistencies

If serum coated, charcoal swabs, expired swabs, or other unsuitable swabs/media are received notes must be made in the item exam of the affected swabs detailing the type of swab submitted. An analytical note must be added for swabs that are received in transport medium.

Refer to appendix 8.3 for the workflow of presumptive/screening testing of SAIKs.

#### 4.2 Acid Phosphatase (AP) Positive Fabrics

Refer to appendix 8.3 for the workflow of presumptive/screening testing of AP positive fabrics.



AP positive fabrics are submitted by QPS. The AP positive area/s should be clearly marked on the fabric, refer to the QPS images for guidance if necessary. If the fabric is not marked then the entire item should be sampled, including both sides of fabric.

AP positive fabrics should be submitted with an additional area surrounding the circled AP positive area to enable the examining scientist to safely hold the fabric during sampling. When a large AP positive fabric has been received it may be necessary to divide marked area/s into sections for separate sampling. Examining scientists are to liaise with the senior scientist if a fabric requires more than 3 samples to be taken.

Images of AP fabrics must clearly indicate the side that is being photographed (e.g. side A or B). If a fabric has no marked areas and both sides of the fabric cannot be visually differentiated, the examiner should label or mark the fabric so sides can be easily identified if a further examination is required. If a fabric contains a seam which can be identified as the I/S or O/S, this should be noted in the item exam.

The entire marked area must be sampled no matter the sampling technique (scraping, excision, tape lifting or swabbing). **Extreme care must be taken during sampling to avoid sharp related injuries**.

Refer to appendices 8.5 and 8.6 for standard labelling of AP positive fabrics.

#### 4.3 Semen in-tubes

Refer to appendix 8.3 for the workflow of presumptive/screening testing of semen in-tubes. All in-tubes that require semen testing are to be examined by scientists only. If semen intubes are stored in an in-tube box, they must be transferred to an items box and added to the examination worklist. If an in-tube check has been completed the tube must be stored to an ERT-AS box and transferred to an examination bench. If an in-tube check has been performed but has not been validated it must be validated by the scientist performing the examination. Three scenarios are as follows:

- In-tube contained within CSSE, no in-tube check performed
- In-tube removed from CSSE, in-tube check performed
- In-tube contained within CSSE, in-tube check performed
- 1. Track ERT-AS box, the in-tube or CSSE to an examination bench
- Check the image/s of the CSSE to ensure the details match the FR
- 3. Remove the tube from the CSSE if necessary, sign and date the opening
- Create an item exam as per appendix 8.3 and describe the tube and contents, note any staining visible if the item is a swab and if a tape lift has been received note whether it appears used or not.
- 5. No tube lot number is required on the item exam as the sample will be submitted in the original in-tube. Scan the ERT-AS box into the storage box ID field.
- 6. If the sample received is a swab, all the swab material is to be cut from the stick and the stick disposed of.
- Follow steps 4 9 in section 4.12.6.
- 8. Refer to section 4.18 to add a result line.



#### 4.4 Condoms

Refer to appendix 8.3 for the workflow of presumptive/screening testing of condoms.

A condom should be described in terms of "O/S surface as received" and "I/S surface as received". Describe any fluid that may be present on or within the condom. Measure the length and diameter and describe any damage, colour, patterning and translucency.

Collect one wet and one dry swab from the O/S and I/S surfaces of the condom. If fluid is visible within the condom then only a dry swab is needed for the I/S surface. Combine the I/S wet and dry swabs into one tube and the O/S wet and dry swabs into another tube.

Note: When sampling the swabs, to ensure that there is not excess substrate submitted, sample the entire wet swab material, but only submit the outer layer of the dry swab.

#### 4.5 Sanitary Pads and Tampons

Sanitary pads are AP tested on the side worn in contact with the skin.

The body of a tampon is cut through the middle and splayed out. The outer sides that were in contact with the skin are to be AP tested, including the string.

#### 4.6 Post-mortem Samples

Refer to appendix 8.3 for the workflow of presumptive/screening testing of post-mortem samples.

PM samples may include sexual assault swabs and/or slides (high vaginal, low vaginal, vulval etc.), pubic hair, head hair, fingernail clippings or scrapings. The testing requirements are to be confirmed by QPS prior to sampling.

Refer to QIS 34300 Examination of post mortem and associated samples from deceased persons, for further detail on post mortem examinations.

#### 4.7 Clothing and Bed sheets

Refer to appendix 8.2 for different scenarios and required result lines for whole item AP testing.

For large items, an examination strategy should be formulated based on the case history and if necessary, in consultation with the QPS. This must be recorded in the item exam or as a notation in the FR.

If the case history suggests that the item has been washed, then it may be necessary to perform microscopy only considering the water-soluble nature of AP and p30.

When describing the I/S, O/S, right side or left side of a garment; examiners should be aware that these terms are used in relation to 'as would be worn'.

#### 4.8 Wet and Dry swabs – QPS submitted

When wet and dry swabs are received from the same site (e.g. high vaginal swab from a SAIK, an item) submit each of the swabs separately.

#### 4.9 Multiple Presumptive/Screening Tests

Consideration should be given to the order in which screening tests are conducted in order to conserve the possible biological material on an item. Where both AP and Phadebas screening tests are required, perform Phadebas testing on the exhibit first (using

Queensland Government commercial paper), once the Phadebas test is complete the Phadebas paper can be sprayed with AP reagent.

#### 4.10 Penile Swabs

The presence of spermatozoa on penile swabs is not unexpected. These swabs are generally submitted for cells only, however where the case history indicates multiple offenders, or the female has had previous sexual contact, they should be submitted for diff lysis with no semen screening performed.

Samples that are submitted straight for diff lysis only that do not require semen screening must have an analytical note "Quant and Amp on SFRAC and EFRAC" (refer to section 4.13).

The diff slide is not read for these samples, microscopic process notes are to be removed and replaced with 'slide not read at this time' should be added to the microscopic process.

#### 4.11 Lubricant Testing

If the lubricant box has been ticked on the SAIK paperwork, QPS must indicate whether lubricant testing is required prior to examination of the SAIK. The SAIK must be placed on hold and a request/task must be sent to the forensic officer or SSLU by the validator or examining scientist, refer to QIS 33771 to create and complete a request/task\_and QIS 33800 for standard wording. **Note:** Lubricant testing cannot be performed if Phadebas supernatant testing is also required. Refer to section 4.19 for the lubricant process.

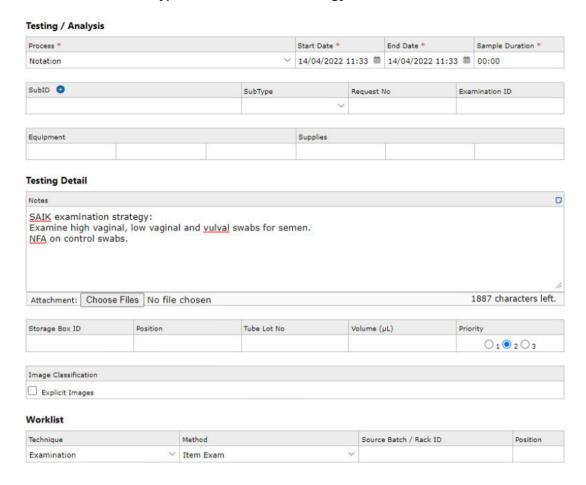
#### 4.12 SAIK examination

#### 4.12.1 SAIK examination strategy

- 1. New SAIKs will be listed on the received worklist, click on the DNA icon and select worklist to view the received worklist. New SAIKs will be tracked to freezer shelf 2 (FDNA-EXFZ-0002) and the SAIK paperwork will be located in the ER in-tray.
- 2. On any page, click the key identifier search icon and scan the barcode attached to the SAIK paperwork, press enter or click search. In the files table click the open the FMO notes pdf. The download box will appear in the bottom left corner. Compare the physical and electronic notes to ensure all pages are scanned, scan and upload any missed pages to a case file notation (refer to appendix 8.7). If a case file notation has been created by FPP but no scanned notes are present, FPP will need to be contacted ( ) to upload the notes.
- Review the FMO notes and ensure any identifiers on the paperwork match the FR
  exhibit record and forensic case file record pages. Ensure the patient the SAIK was
  collected from is visible on the exhibit record page in the description or location/owner
  fields.
- 4. Scroll to the exhibit analytical/testing table on the exhibit record page and ensure the priority is listed as either P1 (if requested) or P2, if the SAIK is listed as P3 a request/task will need to be sent to SSLU requesting that the priority be changed.

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- Click the create exhibit test icon in the exhibit analytical/testing table and select a notation from the process field.
- 6. In the notes field type the examination strategy for the SAIK.



#### Figure 1 – SAIK examination strategy

- 7. In the worklist table select Examination from the technique dropdown menu and Item Exam from the method dropdown menu.
- 8. Click on the save button.

#### 4.12.2 Validation of SAIK examination strategy

- 1. The examination worklist will indicate SAIKs that require examination.
- Click on the exhibit number of the SAIK to be examined, navigate to the exhibit record page and read the FMO notes, which can be found in the "Case Management Reports" table.
- 3. Scroll down to the exhibit analytical/testing table, find the relevant notation and click the date/time hyperlink.





#### Figure { SEQ Figure \\* ARABIC } - Hyperlink to notation

- Read the SAIK examination strategy in the notes field. If you do not agree with the SAIK
  examination strategy discuss with the scientist who created the strategy, they can edit
  the original notation if necessary.
- 5. Once you are satisfied that everything is correct and you agree with the SAIK examination strategy click the edit button.
- 6. In the notes field add an additional note to agree to the examination strategy.

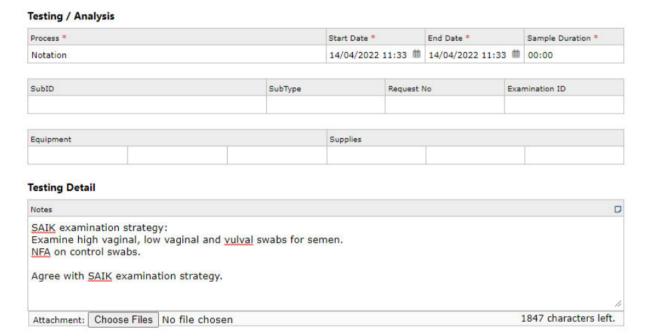


Figure { SEQ Figure \\* ARABIC } - Checking SAIK examination strategy

Click on the save button.

#### 4.12.3 Description of SAIK packaging

- 1. Retrieve SAIK from the freezer location and track to an examination bench in the evidence recovery laboratory via exhibit movement.
- Photograph the packaging and upload images to I:\FR Images.
- 3. Scroll down to exhibit analytical/testing table, click the create exhibit test 🖍 icon.
- 4. In the Testing/Analysis table process field select Item Exam from the dropdown menu.
- In the packaging and sample assessment notes field tick the "Seal and Packaging Intact" box if this is the case. If the packaging and seals are not intact use the notes



- field to describe the nature of the packaging and seals. **Note:** The "Sample meets requirements" box is specific to in-tubes and is not to be used for Item Exam's.
- 6. It is standard practice for FPP to open SAIK packaging to retrieve paperwork prior to delivery to Forensic DNA Analysis. Note whether the packaging has or has not been opened including if it has been re-sealed, signed and dated. Describe any labelling on the SAIK packaging, it is acceptable to state "labelled as per images". List the contents of the SAIK and for each item state whether it is to be examined or not. If all contents are labelled with identical printed labels, this can be detailed in the notes field and referred to in each of the subsequent examination notes.

Testing / Analy	/515			10				
Process *				Start Date *		End Date *		Sample Duration *
Item Exam			Y 14/04/2022	11:40 🏛	14/04/2022 11:4	40 =	00:00	
SubID 💿			SubType		Request I	No	Exa	mination ID
				~				
Equipment				Supplies				
esting Detail								
Packaging and San	mple Assesment	t Notes						
Packaging mate	ches Exhibit ima	ige	Seal and Packag	ing Intact		Sample meets	require	ements
Swab Notes				Cigarett	e Butt Note	es		
Staining	Inten	sity	Sampled	Туре	Type Condit		ondition	
Full  3/4 Swab  1/2 Swab  Tip  None	□ ме	ght edium eavy	Full  1/2 Swab  Stained area	Hand	Hand Rolled (NF) Hand Rolled (F)  B F		Smok Burnt Staini Flatte Stubb	ing ened
Notes								
or dated. QPS Property	Tag attache n opened, re as per imag :: ginal swab – inal swab –	d to front of e-heat sealed es. examined examined	d to be intact as pe SAIK with sticky ta I; signed and dated	pe.	n by exa	miner, tamper (	evide	nt seal not sign
Attachment: C		No file chos	1950.0				10	1598 characters

## Figure { SEQ Figure \\* ARABIC } - Description of SAIK packaging

7. Click on the save button.

#### 4.12.4 Description of SAIK contents and image upload

1. Click the Examinations tab.



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- Click the add button.
- 3. Change the start time to a time before images were taken. In the duration field add an estimate time for the examination.



Figure { SEQ Figure \\* ARABIC } - Examination Record Time and Duration fields

4. The following check boxes must be ticked as this is required for compliance with software requirements: Examination location – General, Recording Method – Photo General (can tick Photo Explicit if images are of a sensitive nature) and No Case File.

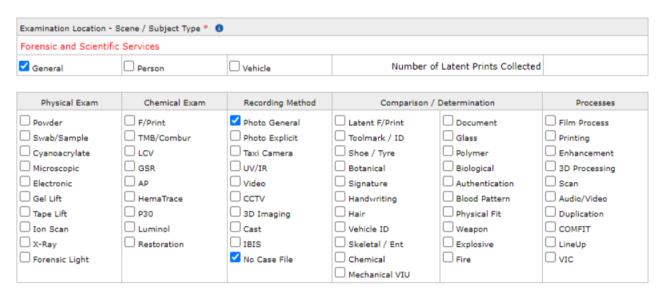


Figure { SEQ Figure \\* ARABIC } - Examination record check boxes

- In the Examination notes field type a summary of the SAIK contents including items not examined such as unused swabs etc. Note: Abbreviations such as HVS or LVS cannot be used. Do not state "examined" or "not examined".
- 6. In the exhibits examined field, scan the SAIK barcode.



Figure { SEQ Figure \\* ARABIC } - Description of SAIK contents



- 7. Click on the save button.
- 8. Click the arrow icon next to the edit button and select upload files/images.
- 9. Alternatively scroll to the images table and click the upload images plus icon.
- 10. The examination file upload box will open, click the add files button

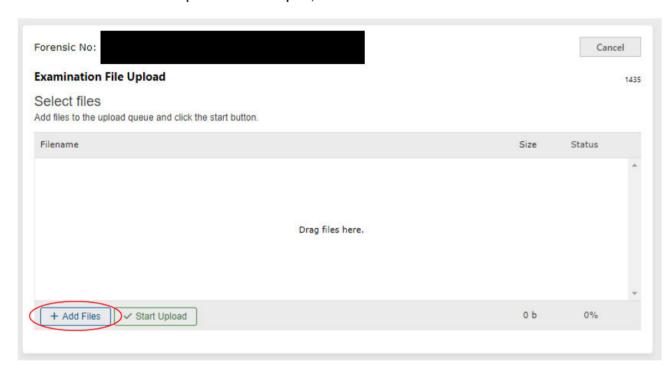


Figure { SEQ Figure \\* ARABIC } - File Upload table

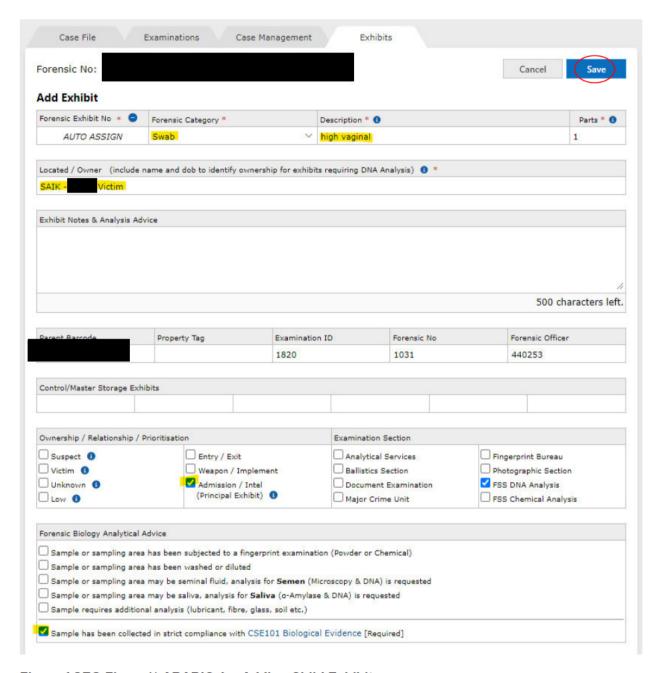
- 11. Navigate to I:\FR Images and find the relevant packaging images. Multiple images can be selected by holding down the Ctrl button. Click open.
- 12. Click the start upload button.
- 13. Once the images have uploaded click the save button.

#### 4.12.5 Registration of SAIK contents - creating child exhibits

- 1. Click the arrow icon next to the edit button and select add related exhibit.
- 2. Click the plus oicon in the forensic exhibit no field to auto assign a new barcode.
- 3. Choose the forensic category (e.g. swab).
- 4. Type in the description (e.g. high vaginal) Note: Abbreviations must not be used.
- 5. The Located/Owner field will auto fill from the parent item, any information that is not required can be removed. Any additional details in the description field of the parent item must be manually transferred. The located/owner field should indicate ownership, for example "SAIK – name of complainant".



- 6. The parent barcode field will auto fill.
- 7. Tick the Admission/Intel and Sample has been collected in strict compliance with CSE101 Biological Evidence boxes.



### Figure { SEQ Figure \\* ARABIC } - Adding Child Exhibits

- Click on the save button.
- 9. To add more child exhibits, click on the back button.
- 10. Click the plus icon in the forensic exhibit no field to auto assign a new barcode and edit the category and description of the 2<sup>nd</sup> child exhibit.
- 11. Click on the save button.



12. Repeat steps 9 – 11 for every component of the SAIK.

These steps are not required for SAIK components that do not require examination, for example control swabs.

Note: To add an additional child exhibit after completion of the above steps, return to the Exhibit Record page for the exhibit, open the examination from the Examinations table, click Edit the arrow icon next to the edit button and follow the steps above.

#### 4.12.6 Examination of SAIK swabs

- For each item in the SAIK perform the item exam procedure. Refer to SOP 33800. Examination of Items for detailed procedures. Ensure to add:
  - a. The Tube Lot number and Storage Box ID (position will autofill)
  - b. Any labelling present
  - c. Sampling details

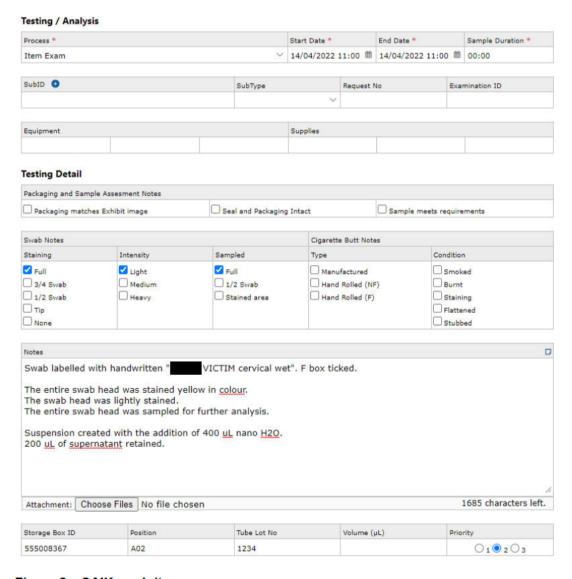


Figure 2 - SAIK swab item exam



- 2. Perform any necessary TMB presumptive testing as needed.
- 3. Place sample in a 1.5ml tube (for diff lysis) or 2mL tube (for cells) and label.

Note: Steps 4 – 9 are for samples that require semen screening only.

- 4. Add 400uL of nanopure water to the tube to form a suspension
- 5. Vortex mix thoroughly
- 6. Incubate on a hot block at 30°C for 15 minutes.
- 7. Vortex mix the sample and spin using the centrifuge for 3 minutes.
- 8. Register a 'RETAIN' subsample and print a tube label.
- Pipette 200uL of supernatant and transfer to a new 1.5ml tube and store frozen in the p30 supernatant box 1.

#### 4.13 Analytical notes

An analytical note e.g. "extract & hold EFRAC" must be added to each sample if required. For "quant & hold" samples submitted for diff lysis (e.g. cold case samples) it must be specified in the analytical note that this applies to both the SFRAC and the EFRAC.

 Scroll down to the exhibit analytical/testing table and click the create exhibit test icon.



- 2. In the Testing/Analysis table process field select Analytical note from the dropdown menu.
- 3. Use the notes field to type the comment for the analytical team.

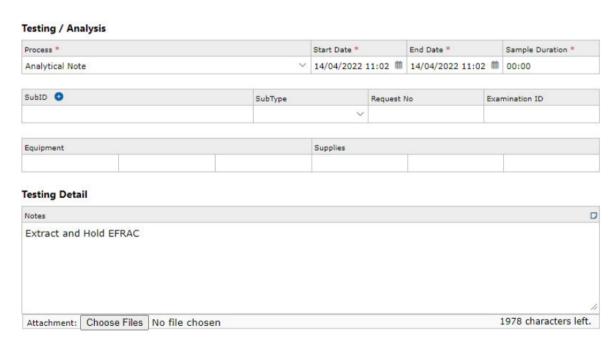


Figure { SEQ Figure \\* ARABIC } - Analytical note



Click on the save button.

**Note:** Analytical notes will auto-validate and do not require validation from a second scientist.

#### 4.14 Drop Sheets

Drop sheets need to be registered regardless of whether they appear used or not. If the drop sheet is marked "not used" (or similar), registration or examination is not required.

- 1. Ensure the category is 'Paper' and the description is 'Dropsheet' when registering.
- Add an item exam, include any packaging and labelling details as appropriate, state
  what is visible on the drop sheet if it appears used or unused. State if any possible
  hairs are present "No possible hairs present NFA".
- 3. If any possible hairs are visible, they must be collected and placed into a CSPB, a 'MISC' subsample must be created (refer to 33800 for creation of subsamples) and the CSPB labelled with the subsample barcode. The number of possible hairs collected must be noted in the item exam and subsample notes field.
- 4. Note in the item exam that the CSPB containing possible hair/s has been retained within the dropsheet CSPB.
- 5. The result line 'HAIRNFA: Hair located not examined as this time' must be added to the dropsheet barcode.

#### 4.15 Finalisation of SAIK examination

Once the SAIK examination is complete, repackage, seal, sign date and track the SAIK to the freezer returns location (FDNA-RTFZ-0001) or to freezer box 4 (FDNA-EXFZ-0004) for lubricant testing.

Store all samples to an ERT-AS box if not already stored during examination, track the ERT-AS box to FDNA-ERER-0001 and place into the hatch for collection.

#### 4.16 Microscopy of diff slides

Refer to QIS <u>17189</u> (Examination for and of Spermatozoa) for staining and examination of microscopic slides.

Refer to Appendix 8.4 for the diff slide process.

#### 4.17 p30 testing

If microscopy is negative for spermatozoa, add a new exhibit test and select 'Presumptive' and select the p30 supernatant subsample barcode from the dropdown list in the SubID, type "p30 required" into the notes field and click save.

**Note:** P30 testing is not required on AP positive fabrics that have been sectioned and one or more sections are positive for spermatozoa. Add a notation "p30 not performed as alternate section <br/>
section > is micro pos for sperm".

1. Click the DNA icon and select "Worklist", click the "Worklist" tab and select "Awaiting Review", "Presumptive".

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- 2. Click and open only the presumptive tests created by the person performing p30 testing as other presumptive tests from examinations may appear on this list.
- 3. Refer to QIS 17185 to perform the p30 test

**Note:** If a p30 kit is faulty (e.g. control line doesn't appear) create the presumptive record but don't select a result. Enter the batch number of the p30 kit in the reagent field and add a comment in the notes section.

- 4. Edit the presumptive and add the p30 reagent code into the reagents field, select the appropriate radio button and remove the comment in the notes field.
- 5. Follow section 4.18 and refer to appendix 8.1 to add and select an appropriate result line.

Create an exhibit movement for each used p30 supernatant tube and discard.

- 1. Navigate to the Forms/Toolbox icon and select "Batch Move Exhibits"
- 2. Scan each supernatant tube barcode into the "Exhibit Movement" table.
- 3. In the location field select "DESTROYED".

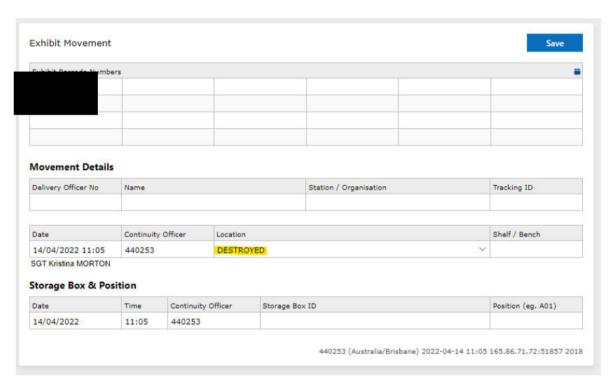


Figure { SEQ Figure \\* ARABIC } - Batch Exhibit Movement

4. Click on the save button.

A box audit must be performed on the p30 supernatant box 1 each week to ensure samples have not been missed.

Positive microscopy p30 supernatants are to be transferred to appropriate month box to be stored for 3 months.



On the first day of the month the person rostered on slide reading is to audit the oldest p30 retain supernatant box and send tubes to "DESTROYED" as per steps above. If tubes are present within the box that have had a NWQPS result line added prior to extraction the p30 supernatant tube must be transferred to the long term freezer storage box located in freezer box 3 (FDNA-EXFZ-0003).

#### 4.18 Result lines

Add a new exhibit test to the exhibit analytical/testing table and select "Result", use the drop-down menu to select the appropriate result line and click save (refer to Appendix 8.1 and 8.2).

#### 4.19 Lubricant testing

1. If QPS have confirmed the SAIK requires lubricant testing, each child exhibit must have an Analytical note added during examination that states:

"All substrates[swab/tapelift/scraping/excised], spin baskets, supernatants and any remaining tubes (that do not contain DNA extract) need to be retained and returned to ER to be submitted to Trace Evidence for lubricant comparison examination".

- All samples for lubricant testing must be added to the extraction worklist "Diff Lysis
  Retain Supernatant". The SAIK must be tracked to freezer box 4 (FDNA-EXFZ-0004)
  after the examination.
- 3. Each Wednesday, the Scientist rostered on supernatant testing must check the Trace Evidence worklist and freezer box 4 for samples that require lubricant testing, they are responsible for packaging the samples for transfer.
- 5. Retrieve the storage box from Analytical and the SAIK from freezer box 4 (FDNA-EXFZ-0004) and transfer both to an examination bench.
- 6. Perform a subsample movement for the 'SUPNAT' and 'SPIN' barcodes relating to each swab site from the SAIK and track to swab site from the SAIK and track to
- 7. Package all components of each swab site in a CSPB together, label each CSPB with the child exhibit barcode. E.g. all tubes relating to the high vaginal swab are packaged together and the CSPB labelled with the high vaginal barcode.
- 8. Take a photo of the sealed and labelled CSPBs for each swab site and upload the photo to a notation on the SAIK barcode. All individual CSPBs can be photographed together as long as the label of each is clear.
- 9. Place individual CSPBs into a larger CSPB, seal with evidence tape, sign and date the seal and label with the SAIK barcode.
- Create a notation under the SAIK barcode and describe the packaging and the contents.



- a. Example for a high vaginal swab: 1 x tube labelled SUPNAT XXXXXXXX, 1 x tube labelled SPIN XXXXXXXXX, 4 x tubes labelled with barcode XXXXXXXX from the high vaginal swab packaged together for lubricant testing.
- 11. Open the SAIK packaging and place the large CSPB inside the SAIK packaging, re seal the SAIK following standard procedures.
- 12. If there are extraction control tubes stored amongst the SAIK tubes within the storage box, these will need to be sent to Forensic Chemistry. Only the 'SUPNAT' subsample will be stored within the box, this sample will need to be converted to an exhibit for tracking purposes. Follow step 8 of appendix 8.3 to convert a subsample to an exhibit.
- 13. Place the extraction control supernatant into a 5mL tube and label, place the 5mL tube into a HSPB, heat seal, sign, date and label. Enter a notation on the negative extraction control to state 'supernatant XXXXXXXX sent with SAIK XXXXXXXX for lubricant testing'. Add a notation to the supernatant barcode and detail the packaging and contents, upload an image of the packaging.
- 14. Attach the HSPB containing the negative extraction supernatant to the SAIK packaging with staples.
- 15. Enter the SAIK barcode and negative extraction supernatant barcode to the exhibit transfer manifest form QIS 36268. Print and complete the packaged by field, another Scientist must review the contents and complete the exhibit list reviewed by field on the form. Scan and upload into FR the transfer manifest form as a case file notation on the SAIK (refer to 8.7). Attach the exhibit manifest form to the SAIK packaging with staples.
- 16. Track the SAIK and the negative extraction supernatant HSPB to freezer returns (FDNA-RTFZ-0001). Send an email to

to alert FPP that samples are located in freezer returns that are ready to be collected to be forwarded to Forensic Chemistry for lubricant testing. Upload the email correspondence as a case file notation to the SAIK in FR.

- 17. If the SAIK requires urgent transport to FPP the SAIK and negative extraction supernatant must be tracked to the DNA storage bench within Forensic Property Point (FPPB-DNAT-0001). Call FPP (3096 2962) to notify them of the incoming SAIK for forwarding to Forensic Chemistry and lubricant testing, deliver the SAIK to FPP.
- 18. Once the SAIK has been tracked to the appropriate location the samples must be reallocated from the Trace Evidence worklist. Click into each barcode on the worklist, add a new exhibit test and select reallocate, ensure the supernatant barcode is entered into the SubID field.

#### 4.20 Fluid samples

If a fluid sample is received for testing for semen (e.g. oral rinse), the following procedure should be followed.

1. Transfer the fluid from the original container to a 1.5mL tube.

**Note:** Depending on the volume received multiple 1.5mL tubes or 50mL falcon tubes may be necessary.

2. Create a balance tube and centrifuge the sample for 3 minutes. If a falcon tube is used it must be transferred and centrifuged in the Analytical laboratory.



- 3. Carefully remove the supernatant from the tube without disrupting the pellet and return to the original container.
- 4. If a falcon tube is used, transfer the pellet using a single use pipette to a 1.5mL tube. Alternatively the pellet can be collected using a swab.
- 5. Follow steps 4 9 in section 4.12.6.



#### 5 Associated Documentation

- QIS: 17185 Detection of Azoospermic Semen in Casework Samples QIS: 17186 The Acid Phosphatase Screening Test for Seminal Stains
- QIS: 17189 Examination For & Of Spermatozoa
- QIS: 17190 Tetramethylbenzidine Screening Test for Blood
- QIS: 22857 Anti-Contamination Procedure
- QIS: 23849 Common Forensic DNA Analysis Terms and Acronyms
- QIS: 33771 Examination of in-tube samples
- QIS: 33773 Procedure for Profile Data Analysis using the Forensic Register
- QIS: 33800 Examination of Items
- QIS: 33998 Phadebas Test for Saliva
- QIS: 34006 Procedure for the Release of Results Using the Forensic Register
- QIS: 34300 Examination of post mortem and associated samples from deceased persons
- QIS: 36268 Exhibit Transfer Manifest

#### 6 References

AS2243.1:2005 Safety in Laboratories Part 1 - General

Workplace Health and Safety Act 2011

Workplace Health and Safety Regulation 2011

Workplace Health and Safety Advisory Standards - various

Health, safety and wellbeing | HSQ staff site



## 7 Amendment History

Version	Date	Updated By	Amendments
1	10/06/2016	A. Houlding	First issue.
2	14/06/2017	A. Ryan	Added examination summary, upload of images for SAIKs and creating related exhibits for each component of the SAIK. Added analytical notes. Added appendices 6-10
3	14/07/2017	A. McNevin	Updated information on required fields for an Examination, information on diff slide process
4	13/12/2017	A. McNevin	Minor edits to reflect current practices in FR; updated screen shots and associated documents
5	02/11/2018	C. Savage	Amendments to Appendices, Updated examination guidelines for SAIKs, other minor wording adjustments to reflect current processes as discussed in team meetings.
6	31/08/2020	S. Byrne & A. McNevin	Added additional information on numbering of areas, other minor wording adjustments, removed "Forensic Register" from title of document, further information on lubricant testing.
7	23/02/2021	A. Ryan	Changes in process following implementation of project#181. Appendices updated.
8	22/04/2022	K Morton	Updated template, added a process for examining fluid samples, semen in-tubes and case file notation creation. Updated lubricant and p30 processes, associated documents, references, appendices and screenshots. Updated content to reflect current procedures.

#### 8 Appendices

- 1 Appendix 1: SAIKs, PM Intimate Exhibits, Semen In-Tubes and AP Fabrics
- 2 Appendix 2: Fabrics/Clothing requiring AP testing
- 3 Appendix 3: Workflow for items semen testing
- 4 Appendix 4: Diff slide process
- 5 Appendix 5: Standardised wording for describing sides of an exhibit
- 6 Appendix 6: Standardised wording for describing subsamples
- 7 Appendix 7: Creating a case file notation



# 8.1 Appendix 1: SAIKs, PM Intimate Exhibits, Semen In-Tubes and AP Fabrics

Table { SEQ Table \\* ARABIC } - Semen testing result lines and processing workflow

Table { SEQ Table \* ARABIC } – Semen testing result lines and processing workflow					
Scenario	Result Line/s	Manual /	Quant/Cease		
		Automatic	testing		
		Result			
Micro pos	Micro positive for sperm – submitted results pending	Manual	Quant		
Micro neg, p30 pos	Presumptive PSA test positive – submitted results pending	Manual	Quant		
	Micro neg for sperm	Automatic			
Micro neg, p30 neg	Micro neg for sperm	Automatic	Cease testing		
	Semen not detected	Manual			
Micro not performed (slide broken), p30 pos	Presumptive PSA test positive – submitted results pending	Manual	Quant		
Micro not performed (slide broken), p30 neg	Semen not detected	Manual	Quant		
Micro neg, p30 not performed (faulty p30 kit)	Micro neg for sperm	Automatic	Quant		
Micro neg, p30 not performed (fabric sectioned by ER and one section is micro positive)	Micro neg for sperm	Automatic	Quant		

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#### Appendix 2: Fabrics/Clothing requiring AP testing 8.2

Table { SEQ Table \\* ARABIC } - AP testing result lines and processing workflow

Scenario	Result Line/s	Manual / Automatic Result	Quant/Cease testing
AP neg	Presumptive seminal fluid test negative	Manual	Cease testing
AP Pos - Micro pos	Presumptive AP test positive — submitted results pending ^	Manual	Quant
	Micro positive for sperm – submitted results pending	Manual	
AP Pos - Micro neg, p30 pos	Presumptive AP test positive – submitted results pending ^	Manual	Quant
	Presumptive PSA test positive – submitted results pending	Manual	
	Micro neg for sperm	Automatic	
AP Pos - Micro neg, p30 neg	Presumptive AP test positive – submitted results pending ^	Manual	Cease testing
	Micro neg for sperm	Automatic	1
	Semen not detected	Manual	1



#### 8.3 Appendix 3: Workflow for items – semen testing

- 1. Click the key identifier search icon and scan the barcode, press enter or click search.
- 2. Using the exhibit movement table, track item to an examination bench.
- 3. Click thumbnail of CSSE, check image, close the image window.
- Add image/s to the item exam or the examination record. Note: Paint or the FR
  annotation application can be used to annotate images.
- 5. Check testing requirements for biological screening.
- Exhibit analytical/testing table → create exhibit test
  - In the process field select item exam.
  - Tick relevant boxes under packaging and sample assessment notes.
  - Enter notes into the notes field including details of the item, details of any staining and markings and how the item is to be sampled.
  - Save.
- Create subsample of scraping/tape-lift/swab/excision. Exhibit analytical/testing table → create exhibit test
  - In the process field select subsample.
  - In the SubID field select the plus icon to auto assign a new barcode.
  - In the subtype dropdown list select MISC.
  - In the notes field add a description of the subsample.
  - Save.
  - Repeat for any other subsamples.
- 8. Convert subsamples to child exhibits:
  - Click on the exhibits tab.
  - Click the add button.
  - Enter barcode of subsample you have just created in the exhibit barcode field.
  - In the category field select the relevant subsample type.
  - Add description of subsample.
  - In the Located/Owner field copy the relevant description from the parent item. If there is additional information within the "Located / Owner" field of the parent item



which will indicate ownership, e.g. a name, "victim" or "suspect" etc. this is also to be included.

- Add the parent barcode in the parent barcode field.
- Tick the following boxes: admission/Intel, FSS DNA Analysis, and sample has been collected in strict compliance with CSE101 Biological Evidence.
- Add your FR User ID in the Delivery Officer Rego field; press tab and your surname will automatically appear. Select Queensland Health Scientific.
- Save.
- Repeat steps for all subsamples.

**Note:** Samples can be created as part of the Examination Record process as outlined in 4.12.5 above.

- Exhibit analytical/testing table → create exhibit test
  - In the process field select item exam.
  - Enter notes into the note field detailing how much water was added to the tube and how much is retained.
  - Add storage box location and tube lot number.
  - Save.
- 10. Create subsample for the retained p30 supernatant:
  - In the process field select subsample.
  - In the SubID field select the plus icon to auto assign a new barcode.
  - In the subtype dropdown list select RETAIN.
  - In the notes field add a description of the subsample.
  - Add tube lot number.
  - Save.
  - Store tube to p30 supernatant box 1.
- 11. Add result line if required. Exhibit analytical/testing table → create exhibit test ✔
  - In the process field select result and select 'submitted results pending' result from the dropdown menu labelled police report.
  - Save.
- 12. Repeat steps 9 11 for all child exhibits.
- 13. Track exhibit to returns.



#### 8.4 Appendix 4: Diff slide process

#### HP2:

- 1. Locate batches ready for microscopy slide processing:
  - Click on the DNA icon, select "Worklist" and click the "Administration" tab.
  - From the dropdown menu select "Workflow Diary"
  - Click "View History"
  - Change the date to the previous working day
  - Look for batches "DNA Extraction Differential Lysis DNA IQ" or "DNA Extraction Diff Lysis Retain Supernatant". The slides from these batches require microscopy.
- 2. Alternatively, click the equipment and supplies icon and select "Storage Box Search". Enter 'slide transfer' into the storage box description and press enter. This will show any slide boxes containing slides ready for staining.
- 3. Retrieve the blue slide storage box from the extraction sorting hatch. An empty slide box needs to be placed into the hatch for the next batch.
- 4. Track the blue slide storage box to an examination bench.
- Transfer each slide to Evidence Recovery lab FDNA-ERER-0001 and place into a slide carrier.
- 6. Stain the slides using Haematoxylin and Eosin, coverslip and allow to dry on the heat block.
- 7. For each individual slide:
  - Exhibit analytical/testing table → create exhibit test 

     and choose Microscopic.
  - · Add diff slide barcode to SubID field.
  - In the SubType field select "SLIDE" from dropdown list.
  - In the Reagents field add the Haematoxylin and Eosin lot numbers, which can be copied and pasted from "equipment and supplies ☐ icon → Supply Search → Category field type "Haematoxylin" and "Eosin".
  - Save.
- Track the empty blue slide storage box to Evidence Recovery Evidence Sorting room FDNA-ERES-0001. Place the slide carrier into the hatch and attach the relevant laminated sign.

**Note:** The negative control slide does not require staining or a microscopic process, this slide is to be stored by the HP3.



#### HP3:

- Scan slide barcode and check whether the diff slide requires reading. FMO prepared slides that were positive or samples that don't require semen testing will not require diff slide microscopy. Add a note to the microscopic page stating, "slide not read at this time".
- Perform microscopy. Edit the existing Microscopic process with the results of the microscopic examination.
- 3. In the "Equipment No" field enter the equipment number for the specific microscope used. Enter the asset barcode of the microscope into the asset number field of the equipment search page from the equipment and supplies icon to locate the equipment number.
- Use radio buttons to record if "spermatozoa were detected" or "no spermatozoa were detected". Note: If the slide cannot be read (e.g. broken), select 'no result' and enter notes.
- Use the notes field to add specific details of the microscopy and any England finder coordinates.
- 6. Click on the save button.
- 7. Store the slide in a diff slide storage box.



#### 8.5 Appendix 5: Standardised wording for describing sides of an exhibit

The following examples detail the standard wording/labelling for describing various sides of an exhibit such as a piece of fabric.

#### 8.5.1 Marking on one side

A piece of fabric received with a marking on one side, item description states item is of AP positive area.

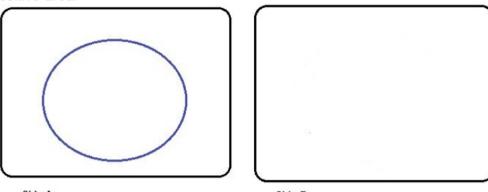


Figure 3 - AP fabric, marking visible on one side

Examination notes should state that the marked side has been designated as side A and the unmarked side has been designated as side B by the examiner.

#### 8.5.2 Corresponding marking on both sides

Piece of fabric received with a marking on one side, item description states item is of AP positive area. Due to material type and / or marker used for marking, the marked area is visible on the other side.

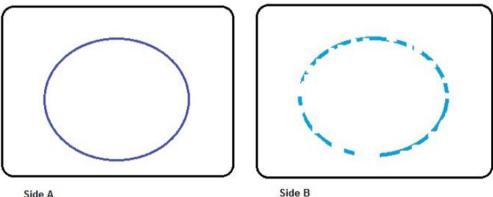


Figure { SEQ Figure \\* ARABIC } - Marked area partially visible on reverse

Examination notes should follow section 8.5.1 and state that the marking from side A appears to have soaked through to side B.

#### 8.5.3 Multiple marked areas on both sides

If both sides of a fabric have markings, the numbering of each marked area will be consecutive and individual for each side.

Example: Side A and side B have multiple marked areas, each area on both sides is to be designated an area number by the examiner. Label side A areas 1 - 3; and side B areas 1 - 3.



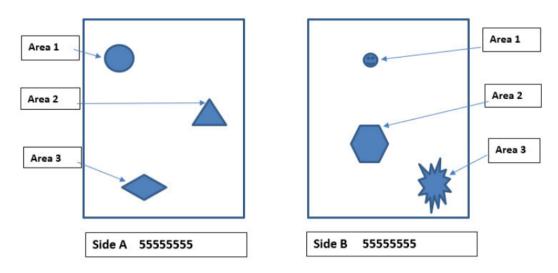


Figure 4 – Numbering of marked areas on both sides of a fabric



#### 8.6 Appendix 6: Standardised wording for describing subsamples

The following examples detail the correct wording to use in the following instances:

- · Where multiple stains / marked areas exist on the same item,
- Where one or more stains / marked areas needs to be divided into two or more smaller sections for sampling.

**Note:** When designating a section, side etc. as per the guidelines below, annotated images is advised.

#### 8.6.1 Creating sections

A piece of fabric that is not marked or the marked area is too big to sample into one tube. Each of the subsamples will be referred to as "sections" i.e. Section 1, Section 2 etc.

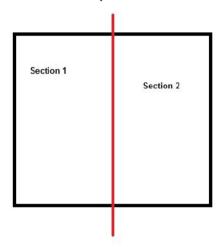


Figure { SEQ Figure \\* ARABIC } - Sections

#### 8.6.2 Multiple marked areas on one side

A piece of fabric that has two marked areas, each area is small enough to be sampled into a single tube each. Each subsample is to be referred to as an "Area" i.e. Area 1, Area 2 etc.

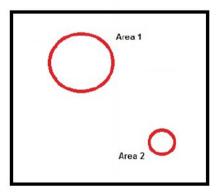


Figure { SEQ Figure \\* ARABIC } - Areas

#### 8.6.3 Multiple marked areas with sectioning

A piece of fabric that has two marked areas, one area sampled as a single subsample, another divided into two sections (too large to sample into one tube).



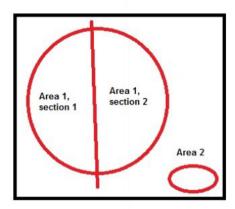


Figure { SEQ Figure \\* ARABIC } - Two Areas, Area 1 two subsamples

#### 8.6.4 Whole item

Follows convention as for piece of fabric above, each area to be referred to as Area 1, Area 2 etc. regardless of whether same or different presumptive result.

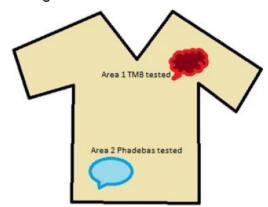


Figure { SEQ Figure \\* ARABIC } – Whole item, different presumptive positive areas

# 8.7 Appendix 7: Creating a case file notation

- 1. Click on the case management tab for the relevant exhibit and click the add button.
- 2. Select the Case File Notation check box in the "Report Type" field.
- 3. Enter the exhibit barcode into the forensic exhibit no field.
- 4. Enter in relevant notes to the comments field (i.e. QP127 scanned).
- Click on the save button.

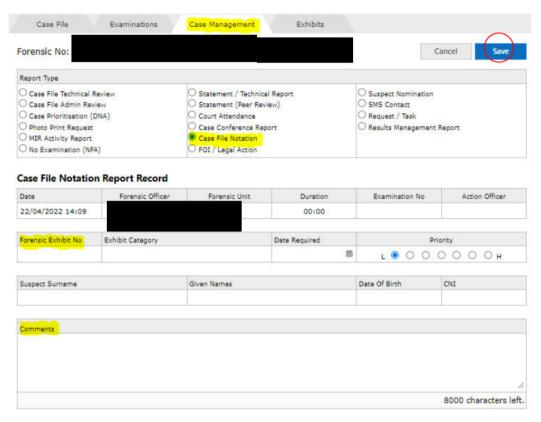


Figure { SEQ Figure \\* ARABIC } - Creating a new case file notation

- 6. In the files table, click the add files plus icon.
- 7. Click "Add Files" or drag the document/s from a folder into the table, click start upload.
- 8. Click on the save button.



# **Queensland Health**

# Forensic and Scientific Services



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#### 1 Purpose

The purpose of this procedure is to describe the processes used for the examination of evidentiary items by Evidence Recovery Scientists and Technicians in Forensic DNA Analysis using the Forensic Register.

# 2 Scope

This procedure applies to all Forensic DNA Analysis staff that examine or interpret examinations of evidentiary items. This standard operating procedure is an adjunct to individual methods for relevant screening tests. Interpretations and limitations of reporting are to be found in each method.

#### 3 Definitions

ERT: Evidence Recovery Team

CSSE: Crime Scene Sample Envelope (packaging used by QPS to store items

collected at a scene

PPE: Personal Protective Equipment SSLU: Scientific Services Liaison Unit SMU: Sample Management Unit QPS: Queensland Police Services

WATB: What appears to be FR: Forensic Register FPP: Forensic Property Point

Dual analysis: The term used for the examination of an exhibit by two or more forensic

sections (e.g. Forensic DNA Analysis and Forensic Chemistry).

#### 4 General Principles

# 4.1 Anti-contamination procedures

QIS document <u>22857</u> describes the anti-contamination procedures for the examination of items, which must be adhered to at all times.

# 4.2 Safety

Full PPE including hair net, safety goggles/glasses, face mask, gown and gloves must be worn in the laboratory for all examinations.

Refer to QIS document 14576 for Forensic and Scientific Services exposure procedures.

## 4.3 Continuity

Continuity is the ability to demonstrate and account for the movements and ownership of an item, meaning that at any point between when the exhibit is seized through to when the exhibit is produced in court or destroyed, its location and all persons who have been in contact with the exhibit can be determined. This provides evidence that the exhibit has not had the opportunity to be tampered with or has not come in direct contact with other exhibits. Refer to QIS document 14077 (FSS- Legal Analysis).

When moving an exhibit or case file, the physical movement must be recorded electronically in the Forensic Register using the exhibit movement function. Depending on the process, this can be done by moving the item from location to location or the storage



Valid From: 17/05/2022 Approver/s: Cathie ALLEN rack or box that the item is contained in. The exhibit movement function must accurately reflect all the locations within the laboratory that the sample has been.

In addition to recording the physical location of exhibits and case files, continuity also includes:

- Recording exhibit packaging details, including seals
- Examination notes
- Use of unique identifying numbers or barcodes for exhibits and subsamples
- Maintaining custody and security of exhibits always. Only items which are drying should be left in the laboratory overnight, all other items must be returned (tracked) to the exhibit room or freezer

#### 4.4 Priority

The QPS will designate a priority for a case and for exhibits, which may differ, case / sample may be given the following priorities:

- Priority 1 (Urgent): Samples specifically approved by the QPS for processing in 3-5 day turn around. Samples may only be processed as Priority 1 with the approval of the Senior Scientist, Team Leader or Managing Scientist. Samples identified as needing to be processed before routine samples, due to an identified specific issue e.g. pending court date for case.
- Priority 2 (High): Allocated based on crime code and generally used for crimes against a person.
- Priority 3 (Medium): Allocated based on crime code and generally used for crimes not against a person i.e. property crime.

The priority of a sample/case may change at any stage and should be reviewed when determining testing or re-testing requirements.

# 4.5 Exhibit notes

The QPS can enter examination strategies or other information to guide the examination by Forensic DNA Analysis in the Exhibit Notes and Analysis Advice field in the Forensic Register.

# 4.6 Dual Analysis

Dual analyses must be completed in the Evidence Recovery laboratory as this location has the optimal environmental conditions for DNA sampling.

Exhibits which are to be transferred to the custody of Forensic DNA Analysis must be receipted as per normal receipting arrangements through FPP. Where the item is maintained in the custody of another section (e.g. when samples are suspected of containing prohibited substances), the chemist will track the exhibit to a Forensic DNA Analysis location but will physically remain with the exhibit.

Where the dual analysis involves hazardous chemicals or other substances (i.e. drugs, explosives etc.) the relevant forensic section is responsible for making a hazard assessment and documenting this as a case file notation. This assessment must include personal risk to staff during examination, storage, subsequent analysis as well as potential risks to equipment.

Refer to QIS 33798 for lubricant procedures.



#### 4.7 Sample Selection

The case history, presumptive/screening test results and the staining present on the item are all used to determine which samples are to be submitted. The following elements should be considered when selecting samples for submission:

- Case history offence type and the modus operandi.
- Number of offenders if there are multiple offenders/complainants then an
  increased number of samples may be required to identify as many involved persons
  as possible.
- Presumptive/screening test results samples of each biological fluid type should be considered for submission.
- Size, location and distribution of staining.

#### 4.8 Sampling techniques

Forensic DNA Analysis uses the following sampling techniques:

- Swabbing
- Tape-lifting
- Scraping
- Excision
- Submission of whole item

**Note:** If the area to be sampled is large, it may be necessary to adopt a checkerboard style of sampling in consultation with the Evidence Recovery Senior Scientist.

**Note:** Invasive/damaging techniques such as excision or scraping should only be used when it is the most appropriate method of recovering DNA and care should be taken as to not cause unnecessary damage to an item/exhibit.

#### 4.8.1 Swabbing – used for non-porous surfaces

Swabs moistened with nanopure water or 70% v/v Ethanol are used to sample the area of interest and the entire swab head is submitted for analysis. In some cases, a dry swab may be used after a wet swab and both swabs combined in one tube for submission.

# 4.8.2 Tape-lifting – used for porous surfaces

The sticky surface of commercial tape is pressed against the area of interest until the tape's adhesive properties are exhausted. Always ensure that a newly exposed section of the tape is used to reduce the chance of contamination. The tape must be rolled with adhesive side in the middle and submitted for analysis in 2mL tubes only.

#### 4.8.3 Scraping

This method is used for fabrics or surfaces where tape-lifting or swabbing are not appropriate and the area of interest is too large to excise. A scalpel handle and blade are used to scrape the top layer of the exhibit.

# 4.8.4 Excision

A scalpel handle and blade are used to excise an entire area of interest that is small enough to fit into a 1.5 or 2mL tube (e.g. ~5mm x ~5mm marked area).

#### 4.8.5 Submission of whole item

This method is used where the entire item as received is small enough to fit into a 1.5 or 2mL tube.



## 5 Pre-examination preparation

Before commencing the examination of an item, all available case details should be reviewed to determine the type of examination and the testing required. This information may also be used to prioritise examinations. The following items should be reviewed:

- Exhibit notes & Analysis Advice field
- Medical notes including SAIK paperwork
- QP127 (if available)
- Relationship/Prioritisation information
- Exhibit description

Information on the parent item may also be viewed if QPS have ticked the FSS DNA Analysis box.

Where the above information does not provide sufficient information to determine testing requirements the following additional strategies may be employed:

- Contacting the Investigating Officer, SOCO or Scientific Officer either directly or through SSLU (refer to QIS 33771 to create and complete a request/task).
- Contacting the QPS DNA Sample Management Unit
- Contacting FMOs or FNEs

All communications must be recorded electronically by a case file notation or request/task.

Note: Specific details relating to the examination of sexual cases are outlined in QIS 33798.

**Note:** Specific details relating to the examination of post mortem and associated samples from deceased persons are outlined in QIS <u>34300</u>.

Note: If an adverse event occurs during any examination refer to QIS 30800.

#### 6 Examination

#### 6.1 Specific examination strategies

Refer to appendices 10.2 to 10.6 for the workflow of items with different scenarios.

#### 6.1.1 Examination of clothing/footwear for epithelial cells

Generally, only a small number of epithelial cells are deposited by touching or wearing items. It is best to use one side of a swab or a piece of tape no more than 2cm long to collect for submission, thereby concentrating cellular material into one sample.

High friction areas, including armpits, collars, inside collarbone, waist bands, hat bands and other parts of clothing that are in constant contact with the wearer are ideal areas to sample.

#### 6.1.2 Swabs

Record the amount of the swab that is stained, the colour, the stain intensity, the result of any screening tests and the amount of the swab that is submitted for DNA analysis. The entire swab head material can be cut off and submitted for testing.

# 6.1.3 Cigarette Butts

When examining cigarette butts, use the cigarette but notes table to select the appropriate check boxes to indicate whether the cigarette appears to have been smoked, whether there



is burnt tobacco or paper, whether it has been stubbed/flattened and any brand names visible on the butt. Select the appropriate check boxes to identify if the cigarette butt is hand rolled (with or without a filter) or a manufactured type. See Figure { SEQ Figure \\* ARABIC } for automatic lines that are used for cigarette butt examinations. When sampling cigarette butts, any tobacco and/or filters present are not submitted for testing.

- Smoked manufactured cigarettes: Excise a 0.5cm circumference of the filter paper from the butt using a scalpel blade and submit for testing.
- Smoked hand rolled cigarettes: Submit entire cigarette paper for testing.

For manufactured cigarette butts, once sampling has been completed, any remaining portion of the filter paper and exposed tip of the filter is retained as a subsample in the item retention box. For hand-rolled cigarette butts, any tobacco and/or entire filter is to be retained as a subsample, no part of a hand-rolled cigarette is to be discarded.

If multiple cigarette butts are contained within one CSSE, complete an item exam for the packaging only, in the notes field state how many cigarette butts are present. Each individual cigarette butt is registered as a subsample and converted to a child exhibit see sections 6.8 & 6.11, alternatively an Examination Record may be created. Refer to QIS 33798 for specific information on creating an examination record.

Submit the entire cigarette paper and filter paper for testing for unsmoked manufactured and hand-rolled cigarettes. If there is too much substrate for one tube, the sample must be submitted for extraction in multiple tubes to be pooled (refer to appendix 10.7 for the pooling process).

If a cigarette butt has a TMB positive stain, two subsamples need to be created and converted to child exhibits. One subsample will consist of any unstained filter paper as would routinely be submitted and the other subsample will consist of the stained portion of filter paper. The presumptive test must be recorded against the parent barcode.

#### 6.1.4 Syringes, Needles and other sharps

#### Packaging and labelling

Syringes should be appropriately contained in a sharps container and labelled prior to receipt. Syringes that are not correctly contained are to be reported by the examining Scientist to the Evidence Recovery Senior Scientist for action. This action should include identifying the non-conformance in a Case file Notation in the FR and possibly raising an OQI.

#### Safety

Safety is important when examining these items as they pose a sharps risk. Syringe and needle analysis must not to be performed by untrained staff unless under the direct supervision of trained senior staff. If there are any concerns about the sampling of a syringe (e.g. feeling unwell) discuss with the Evidence Recovery Senior Scientist prior to commencing examination.

Re-capping of syringes may be required to preserve exhibit integrity post sampling. This is due to the nature of Forensic testing, whereby those areas of interest (inside of cap and outside of needle) are in a contained environment due to the syringe being capped. Preservation of that contained environment post sampling to maintain sampling integrity and limit possible environmental contamination is required. Considerable care and caution should be taken when re-capping syringes. If the recapping of a syringe is not necessary (e.g. when received uncapped) then it should not be carried out.



#### **Procedure**

 Carefully remove the syringe from the sharps container using forceps and place onto a large petri dish.

**Note:** Even if the syringe needle is capped, exercise extreme caution. Always maintain control of the syringe and needle, keep the exhibit low on the bench and close to the petri dish with the sharp facing downward.

- The hierarchy of syringe sampling is governed by the case circumstances. If sampling of both the external and internal surfaces of the syringe are required, then the following order should be adhered to:
  - a. Place a clamp on a section of the syringe that is secure (refer to Figure 1), recommended locations are the tip (circled in orange), barrel flange (circled in blue) and the plunger end (circled in red). Hold the clamp to safely manoeuvre the syringe during sampling.
  - b. Moisten a swab with nanopure water and swab the entire outside surface of the syringe (barrel and plunger) and cap if present.
  - c. If the needle is covered with a plastic cap it will need to be removed for sampling. Face the needle toward the petri dish, hold the clamp on the syringe and place a secondary clamp on the cap. Apply light pressure with a twisting motion to slowly remove the cap. Always use the clamp to manoeuvre cap when sampling.
  - d. Moisten a swab with nanopure water and swab the entire needle and inside of the cap if present. The needle and inner cap can be sampled together.

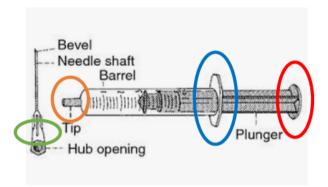


Figure 1 – Parts of a syringe and needle

(Note: There are various configurations of syringes that may be received including syringes with non-removable needles)

- 3. If the syringe contains suspected blood, hold onto the clamp and carefully remove the plunger to sample the contents of the barrel using a swab moistened in nanopure water.
- 4. If there is suspected dried blood in the needle, clamp the hub of the needle (see Figure 1 circled in green) and hold both clamps on the needle and syringe to slowly remove the needle. A swab moistened with nanopure water is then used to collect a sample from the needle, needle/syringe junction or syringe tip. Ensure the needle is secured with a clamp during sampling.
- 5. Note: Step 4 may not be required depending on the syringe/needle type, some needles are unable to be separated from the syringe.
- 6. If a needle is received with no syringe it must be adequately contained upon receipt. A clamp must be used to secure the needle during sampling.



- Upon completion of the examination, safely reapply the cap using the same technique as step 2c.
- Return the syringe/needle to the original packaging. If the item cannot be returned to the original packaging, consider using a larger sharps container to return the contents. Consult the Evidence Recovery Senior Scientist if required.

#### 6.1.5 Possible hairs

If a possible hair is located on an item, the examiner must create a subsample on the exhibit that is being examined. Follow section 6.12 and manually add the result line "HAIRNFA - Hair located – not examined at this time" to the parent barcode. Transfer the possible hair to a clip seal plastic bag, label appropriately and return the item with the original packaging of the parent exhibit.

# 6.1.6 Examination of large volume fluid samples

If a fluid sample is received for testing for cells (e.g. Urine), the following procedure should be followed.

1. Transfer the fluid from the original container to a 1.5mL tube.

**Note:** Depending on the volume received, multiple 1.5mL tubes or 50mL falcon tubes may be necessary.

- 2. Create a balance tube and centrifuge the sample for 3 minutes. If a falcon tube is used it must be transferred and centrifuged in the Analytical laboratory.
- 3. Carefully remove the supernatant from the tube without disrupting the pellet and return to the original container.
- 4. If a falcon tube is used, transfer the pellet using a single use pipette to a 1.5mL tube. Alternatively the pellet can be collected using a swab.
- Submit the tube containing the pellet.

#### 6.2 Tracking of Storage Boxes

- Check shelves (FDNA-EXSH-0696 FDNA-EXSH-0700) in the Exhibit Room (6106B) for item boxes; these are delivered by FPP on a daily basis, Monday to Friday.
- 2. Click the equipment and supplies icon and select "Storage Box Search".



Figure 2 – Equipment and Supplies icon, Storage Box Search

3. In the Storage Box No field scan the barcode of the box and press enter or click submit.



Figure { SEQ Figure \\* ARABIC } - Storage Box table

4. In the Box Movement table click the add storage box movement plus icon.



Figure { SEQ Figure \\* ARABIC } - Box Movement table

 In the Storage Location field, scan the room location from a location sheet or enter "EVI" and from the dropdown list and select "DNA Evidence Recovery Evidence Recovery" (FDNA-ERER-0001).

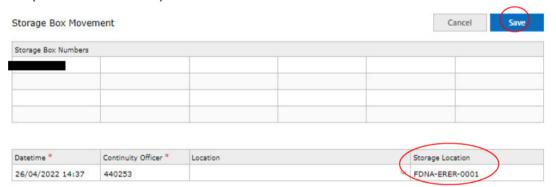


Figure { SEQ Figure \\* ARABIC } - Storage Box Movement, Storage Location

- 6. Click on the save button and place the storage box into the pass-through hatch.
- 7. Complete a new Exhibit Movement following the above steps and track an ERT-AS box to a relevant bench location (FDNA-EREB-0001 0015) before tracking samples.
- 8. Item boxes must be tracked back to the Exhibit Room Returns (FDNA-EXRT-0001) when they have no contents remaining. Follow the above steps to track the box and ensure the contents show as "0/0" on the Storage Box Record page.
- Item boxes must be tracked back to a shelf (FDNA-EXSH-0696 FDNA-EXSH-0700) in the Exhibit Room (6106B) at the end of each day if they still contain exhibits. Follow the above steps to track the box to a shelf.



## 6.3 Assessment of testing requirements

1. On any page, click the key identifier search icon and scan the exhibit barcode and press enter or click search.

**Note:** If the exhibit record is not visible, check the description on the CSSE to ensure that the exhibit is for Forensic DNA Analysis. Contact the QPS Forensic Reception Centre on (0) identify yourself and explain that you have a sample that is not visible in the FR that requires the Forensic DNA Analysis box to be ticked. If the sample is not for FDNA, then it should be returned to QPS untested (refer to section 6.14).

- 2. In the Exhibit Record screen, scroll down and click on the thumbnail image of the CSSE, a larger image will open in a new window. Check the image and item description ensuring all details match the packaging. Check the image quality, ensuring barcodes affixed to the exhibit and other labelling are legible and that the entire CSSE is visible in the image. If the image does not meet requirements, a new photo will need to be taken and uploaded refer to QIS 33771 for details.
- 3. Close the window containing the image.
- 4. Check the testing requirements (see Figure 6) and assess the item to see if biological fluid screening is required before submitting for DNA analysis.

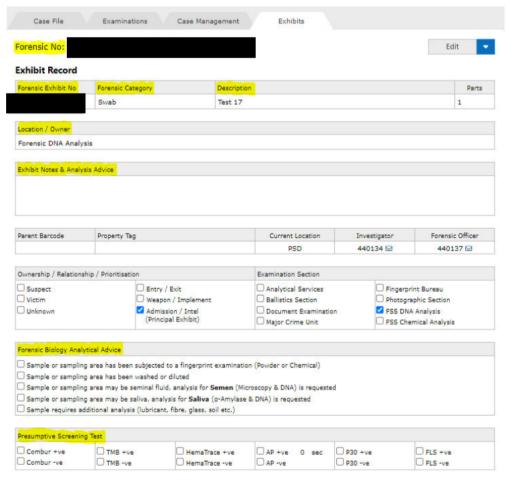


Figure 3 – Exhibit Record page



# 6.4 Digital Imaging

Photos must be taken for exhibits which are complex and/or difficult to accurately describe in typed notes. Smaller, uniform items (i.e. cigarette butts, fingernails, straws etc.) do not require photos, except where there is unusual staining, damage or other features which are difficult to describe. A scale and exhibit barcode must be included in every image. When photographing items with two sides that are designated side A and side B by the examiner, the side that is being photographed (e.g. side A or side B) must be specified and visible in each image. **Note:** Multiple images can be uploaded to an item exam.

If the packaging is damaged in any way, it must be re-photographed. If additional images are required, a new photo will need to be taken and uploaded – refer to QIS 33771 for details. **Note:** All images are stored on the network for 12 months.

# 6.4.1 Annotating images

If images need to be annotated this can be done using the FR annotation application or the windows paint program. Always ensure that the original image and the annotated image are both uploaded to FR.

## Annotating using the FR annotation application:

- 1. Upload the image to the item exam and press save.
- 2. Click on the image in the images table of the item exam and click the "Annotate" button, annotate the image.
- 3. Enter "Annotated image" into the Filename field and ensure a title and description are entered into the annotation details table for each numbered area marked (both can be the same e.g. area 1).
- 4. Press the save button. The annotated image will appear in the Files table field as a pdf.

**Note:** The file/annotated image cannot be edited after it is saved.

# Annotating using paint:

- 5. Save a copy of the image in I:\FR Images.
- 6. Right click on the image > Open with > paint.
- 7. Use the Save As function to save as a JPEG with the original filename and "Annotated".
- 8. Use the functions in the paint program to annotate the image.
- Save the image.
- 10. Upload the annotated image to the item exam.

# 6.5 Packaging

Packaging should be opened in such a way as to maintain the original seals. When
packaging is opened, the staff member must write "Opened" followed by their initials
and date.

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- Scroll down to the Exhibit Analytical/Testing table, click the create exhibit test 
  icon.
- In the Testing/Analysis table process field select item exam from dropdown menu.
- In the Packaging and Sample Assessment Notes table, tick the relevant boxes to describe the nature of the packaging and the seals (refer Figure { SEQ Figure \\* ARABIC }).

**Note:** The Sample meets requirements check box is specific to in-tubes and must not be used for an item exam.

5. If the seals are complex, the tick boxes do not need to be used, the notes field can be used to describe the packaging and seals.

#### **Testing Detail**

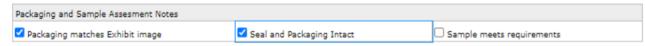


Figure { SEQ Figure \\* ARABIC } - Description of packaging tick boxes

**Note:** Packaging matches exhibit image check box expands to "The packaging matches the QPS exhibit image". This is to be used only when the entire packaging is visible in the QPS image and matches exactly what is received.

**Note:** If multiple exhibits are received in a single package, an Examination Record can be created to describe the packaging once. Refer to QIS 33798 for more details. Each of the exhibits contained within the packaging can then be added to the Exhibit/s Examined table.

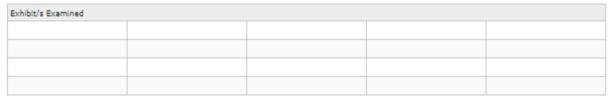


Figure 4 - Exhibit/s Examined fields in Examination Record

#### 6.6 Item descriptions

- The Notes field is used to type the examination notes. If the exhibit is a swab or cigarette butt the text can be auto-generated by ticking the relevant boxes in the Swab or Cigarette Butt Notes tables.
- 2. Exhibits must be described according to the following minimum requirements:
  - What it is
  - Size (including measurements)
  - Labelling/brand
  - Colour
  - Staining (including any presumptive tests conducted)
  - Physical appearance of damage (without commenting on the cause of the damage)
  - Whole items must be further described to categorise the "inside/outside" surfaces and "left/right side" of the garment.



**Note:** When describing the I/S, O/S, right side or left side of a garment; examiners should be aware that these terms are used in relation to 'as would be worn'.

- 3. Staining must be further described according to:
  - Shape
  - Distribution
  - Colour
  - Size (including measurements)
  - Intensity
  - Which side of the item the stain may have originated from
  - Any presumptive tests performed
  - Odour if applicable
  - Whole items should include where the stain is positioned i.e. left/right side of garment as would be worn

Note: Images can be used if the physical appearance of stains are difficult to describe.

Refer to appendices 5 and 6 in QIS 33798 for standardised wording when describing subsamples and sides of an exhibit.

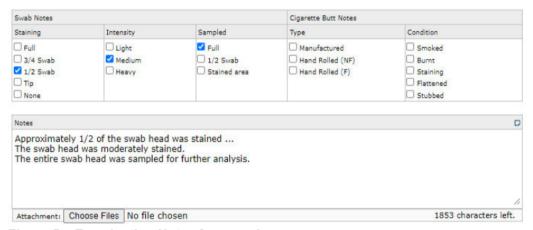


Figure 5 - Examination Notes for a swab

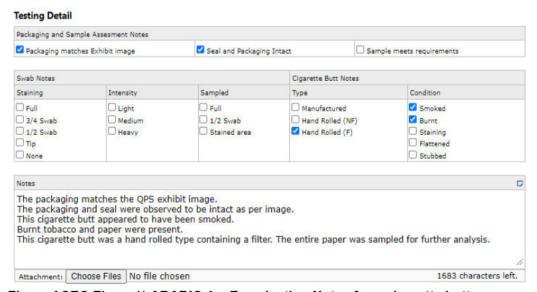


Figure { SEQ Figure \\* ARABIC } - Examination Notes for a cigarette butt



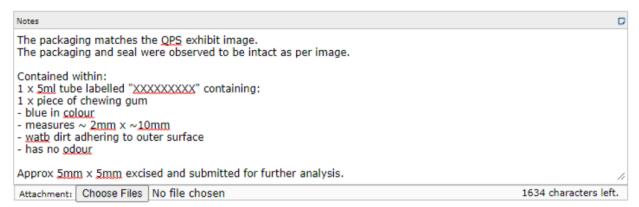


Figure { SEQ Figure \\* ARABIC } – Examination Notes for miscellaneous items

- 4. Scan the ERT-AS box barcode into the Storage Box ID field.
- 5. Scan the barcode affixed to the bag of tubes into the Tube Lot No field.

Storage Box ID	Position	Tube Lot No	Volume (µL)	Priority
	A02	1234		O 1 O 2 • 3

Figure { SEQ Figure \\* ARABIC } - Recording Storage Box ID and Tube Lot No

6. Click on the save button.

**Note:** It is recommended that samples are stored when completing the item exam for each exhibit. Where this is not possible refer to section 6.13.

#### 6.7 Presumptive or Screening tests

If no presumptive testing is required proceed to 6.8. If the examining Scientist elects not to perform a presumptive or screening test, a record of this must be recorded in the examination notes (e.g. if presumptive testing would consume the sample). Where an examination strategy has not been prepared, the examining Scientist is responsible for assessing the exhibit and selecting the appropriate presumptive and/or screening tests.

Forensic DNA Analysis uses the following screening tests:

- TMB test for blood see QIS 17190
- AP test for seminal fluid see QIS 17186
- Phadebas test for saliva see QIS 33998
- P30 test for seminal fluid see QIS 17185.
- Microscopy for spermatozoa see QIS 17189

Note: Results of a presumptive test must only be recorded if a valid control has passed.

#### 6.7.1 Recording details of presumptive testing

Record the details of a presumptive test against the parent exhibit (testing performed prior to sampling) or the child exhibit (testing performed on the subsample).

- 1. Click the create exhibit test \( \ \ \) icon in the exhibit analytical/testing table and select presumptive from the dropdown menu in the process field.
- Record the results by checking the appropriate radio button and use the comments field to make any additional notes.



- 3. Enter the results and if necessary add an annotated image into the exhibit's initial Item Exam. If there is insufficient space, creation of a new Item Exam may be necessary.
- 4. Record details of reagent lot numbers in the Reagents field. The name of the reagent will auto-populate after saving.

#### **Testing Detail**

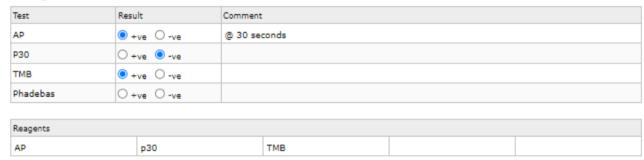


Figure 6 - Presumptive testing detail and reagents fields

5. Click on the save button.

# 6.8 Registration of subsamples

During an item examination, any samples that are created can be registered as a subsample or alternatively examiners can use the Examination Record process (refer to sections 4.12.4 and 4.12.5 in QIS 33798 to create an examination summary record and register related exhibits). Subsample's must be upgraded to an exhibit before being submitted to Analytical (see section 6.11). This upgrade will ensure that a profile analytical detail page is created and results can be reported back to QPS. See section 6.8.2 for exceptions.

- 1. Click the create exhibit test icon in the exhibit analytical/testing table and select subsample from the dropdown menu in the process field.
- 2. In the SubID field click the plus icon to auto assign a new barcode.
- 3. In the SubType dropdown list select MISC.
- 4. In the Notes field add a description of the subsample.



Figure 7 - Subsample registration

- Click on the save button.
- 6. Repeat steps 1 5 for all subsamples as required.
- 7. For analytical processing, convert subsamples to child exhibits refer to section 6.11.

# 6.8.1 Subsamples for retained portions

For portions of a sample that are to be retained (e.g. remainder of filter paper and end of filter of a manufactured cigarette butt), the retained portion must also be registered as a subsample using the following steps.

**Note:** This subsample is for storage purposes only so will not require conversion to a child exhibit.

- 1. Click the create exhibit test \( \infty \) icon in the exhibit analytical/testing table and select subsample from the dropdown menu in the process field.
- 2. In the SubID field click the plus icon to auto assign a new barcode.
- 3. In the SubType dropdown list select RETAIN.
- 4. In the Notes field add a description of the subsample.
- Track the item retention box to the examination bench (see section 6.2) and scan the box barcode to the Storage Box ID field. Alternatively, a group of subsamples can be stored as per section 6.13.
- 6. Scan the label affixed to the relevant tubes into the Tube Lot No field.

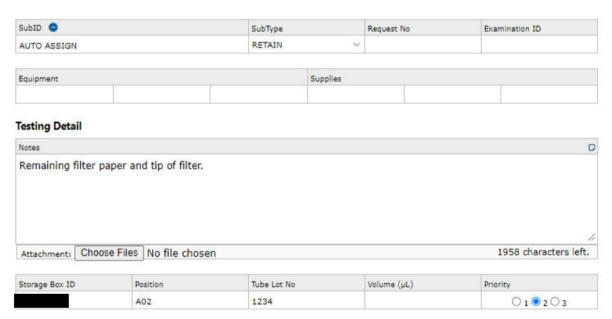


Figure 8 – Registering retained portion of cigarette butt

7. Click on the save button.



#### 6.8.2 Subsamples for ante-mortem exhibits

Ante mortem samples that are collected for the purposes of identification only, will remain registered as subsamples and not be upgraded to child exhibits. For example, tissue samples and toothbrushes etc.

# 6.9 Analytical Notes

- 1. Click the create exhibit test icon in the exhibit analytical/testing table and select Analytical Note from the dropdown menu in the process field.
- 2. Use the Notes field to type an appropriate comment for the Analytical team.
- 3. Click on the save button.

# 6.10 Printing tube labels

1. On the exhibit record page click the arrow icon next to the edit button and select "3 Part Tube Barcode".



Figure { SEQ Figure \\* ARABIC } - Printing sample tube labels

- 2. A new window will open displaying the 3 part label, click the printer icon and select print.
- 3. To print a subsample barcode, click on the subsample hyperlink from the exhibit record page and follow the above steps.

#### 6.11 Converting subsamples to child exhibits

Click on the Exhibits tab and click the add button.



Figure { SEQ Figure \\* ARABIC } - Exhibits tab and add exhibit button

2. Enter barcode of the subsample into the Forensic Exhibit No field.

**Note:** A warning will display when a barcode has already been used (e.g. when upgrading a subsample to an exhibit). The warning appears as:





#### Warning

Warning: This Barcode has previously been allocated to a subsample

# Figure { SEQ Figure \\* ARABIC } - Warning display

- In the Forensic Category field select the relevant subsample type from the dropdown menu.
- 4. Add description of subsample.
- In the Located/Owner field, copy the relevant description from the parent item. If there is additional information within the Located/Owner field of the parent item indicating ownership (e.g. a name) this must be added.
- 6. Add the parent barcode into the Parent Barcode field.
- 7. Tick the "Admission/Intel (Principal Exhibit)" and the "Sample has been collected in strict compliance with CSE101 Biological Evidence [Required]" boxes.
- 8. The examiner must enter their FR User ID in the Delivery Officer Rego field, press tab for the Surname field to auto-populate and select Queensland Health Scientific from the dropdown list in the Station field.
- 9. Click on the save button.
- 10. Repeat steps 1 9 for all subsamples if required.



Forensic No							Cancel
Add Exhibit							
Forensic Exhibit No +	Forensic Cate	gary *	Description * C	•			Parts 1
	ab		inside condom	1			1
ocated / Owner (Inc.)	clude name and dob t	io identify ownership for exhib	its requiring DNA	Analysis)	0.*		
xhibit Notes & Analy	sis Advice						
				20			500 characters
Parent Barcode	Property Tax	g Examinati	on ID	Forensia	: No		Forensic Officer
				1031			440253
Control/Master Storag	ge Exhibits				T		10
Wnership / Relations	ship / Prioritisation		Examination	Section			
Suspect 0		Entry / Exit	☐ Analytica	100000000000000000000000000000000000000	7	□ Eno	erprint Bureau
Victim 0		Veapon / Implement	☐ Ballistics				ographic Section
Unknown 0		Admission / Intel	☐ Documer	nt Examinati	on	✓ FSS	DNA Analysis
Low 0	-0	Principal Exhibit)	☐ Major Cri	ime Unit		FSS	Chemical Analysis
Sample or samplin Sample or samplin Sample or samplin Sample requires as	g area has been subje g area has been wash g area may be semina g area may be saliva, dditional analysis (lub collected in strict comp	ected to a fingerprint examina and or diluted al fluid, analysis for <b>Semen</b> (I analysis for <b>Saliva</b> (a-Amyla ricant, fibre, glass, soil etc.)	Microscopy & DNA ise & DNA) is requ	) is requesti rested	ed		
Combur +ve	☐ TMB +ve	☐ HemaTrace +ve	☐ AP +ve	sec	☐ P30 +ve		FLS +ve
Combur -ve	☐ TMB -ve	☐ HemaTrace -ve	☐ AP -ve		☐ P30 -ve		FLS -ve
Forensic Triage		TOWN SECTION ASSESSMENT	Sample Mar	nagement			
Intel FTA Card		lo Testing Required					
xhibit Warnings			Specific Har	ard Concern	ns .	Storace	e / Handling Requirement
	- return by DD/MM/	YYYY	☐ Sharps H	The second second			sified Item
	ques Not Authorised		☐ Infectiou			Bled	trical Discharge Device
Held - Interim Ord			Chemical				arm (Cleared)
No Comparison Material							arm Related Item of value (e.g. jewellery)
						Drug	
Graphic Warning			☐ Explicit C		. 20.15		gerous Goods
						-	
Film Number	Origin Property P	oint	Origin Prope	erty Tag		Lot / Ba	atch No
	5	El El MESEUL	201				
Delivery Method	Delivery Officer F	Rego * Surname *		Station			
FSS	V 440253	MORTON		Queensla	nd Health Sc	ientific	

Figure { SEQ Figure \\* ARABIC } - Exhibit Record

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11. Any further testing carried out on the child exhibit (e.g. presumptive testing) is to be added to the Exhibit Analytical/Testing table on the child exhibit.

# 6.12 Entering exhibit result lines

Exhibit result lines are created to communicate results to the QPS electronically. Some results will automatically be generated by ticking various boxes or radio buttons; however, some results will need to be entered manually following the steps below:

**Note:** The result lines only appear in the Exhibit Analytical/Testing table (and are autovalidated) after the associated process has been validated. Refer to appendix 10.1 for manual and automatic result lines.

- 1. Click the create exhibit test icon in the exhibit analytical/testing table and select Result from the dropdown menu in the process field.
- 2. In the Police Report field select the appropriate result(s) from the dropdown menu (up to three results can be added at any one time).
- 3. Click on the save button.

#### 6.13 Sample tracking

If samples have not been stored during the examination, they can be added to a storage box at the end.

1. Click the equipment and supplies icon and select "Storage Box Search". Scan the ERT-AS box barcode into the storage box no field and press enter or click submit.



Figure 9 – Storage Box search table

Check that the latest entry in the Box Movement table is the examination bench. If
necessary, click the plus icon and enter "EVI" into the storage location field and select
"FDNA-EREB- (DNA Evidence Recovery Bench) and enter the bench number, press
the save button.



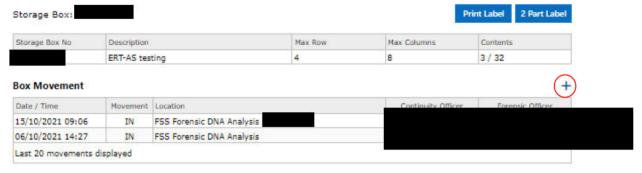


Figure { SEQ Figure \\* ARABIC } - Box Movement table

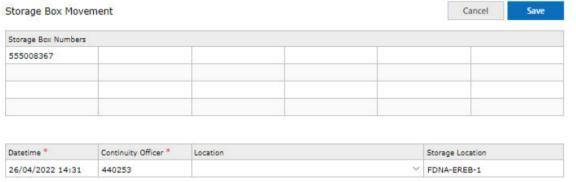


Figure { SEQ Figure \\* ARABIC } – Storage Box Movement

3. Click on the Storage Box Contents tab and click the Add to Storage Box button.

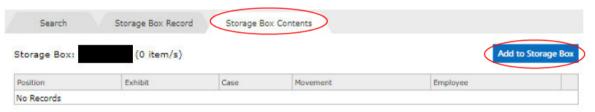


Figure { SEQ Figure \\* ARABIC } - Storage Box Contents

- 4. Scan the barcode of the tube into the Forensic Exhibit No field, note the position in the rack and place the tube into that position.
- Click on the save button or press enter. Repeat the above steps for any further tube storage.



Figure { SEQ Figure \\* ARABIC } - Add to Storage Box table

Follow step 2 and track the ERT-AS box to the generic laboratory location (FDNA-ERER-001), place the storage box in the pass-through hatch.



 The pass-through hatch must be checked each afternoon and any ERT-AS boxes that have not been collected must be stored to freezer box 1 (FDNA-EXFZ-0001), following the procedure in section 6.2.

## 6.14 Exhibit repackaging and return

Exhibits should be repackaged in the same packaging if practical. Re-seal the openings with evidence tape, sticky tape or heat seal and initial and date the seal.

If an exhibit is wet as the result of examination, it can be placed on the drying rails overnight. Ensure that the rails are cleaned with bleach and ethanol before and after drying. Exhibits must have a piece of brown paper between the rail and the item and an additional piece of brown paper covering the item. Ensure that the brown paper is adequately labelled.

If examination of an exhibit is not complete, the item must be tracked back to freezer box 2 or a shelf in the exhibit room. Where the examination is complete, the exhibit must be tracked to the Exhibit Room return location for room temperature samples (FDNA-EXRT-0001) or to the Freezer returns location for frozen samples (FDNA-RTFZ-0001). Exhibits should be stored in the same way they were received (room temperature or freezer).

# 6.15 Temporary storage of CSSE and destruction

Empty CSSE's are placed into a bundle according to the month they are examined, within the items destruction box, which is located in the Evidence Recovery laboratory.

A bundle of CSSE's that were processed 3 months prior must be discarded into a biohazard bin every month.

Note: CSSE's are not to be tracked electronically to the destruction box.

CSSE's that contained multiple cigarette butts require an exhibit movement on the parent barcode (the CSSE). In the location field select "DESTROYED" from the dropdown menu and click the save button.

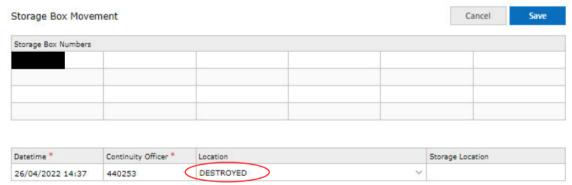


Figure { SEQ Figure \\* ARABIC } - Destruction of CSSE for multiple cig butts in one CSSE

# 6.16 Repackaging of multiple exhibits (subsamples and or child exhibits) into primary packaging

On rare occasions multiple exhibits (subsamples and or child exhibits) may be repackaged into one larger outer package for storage and/or return. In these instances, the individual exhibits cannot be left tracked to an examination bench or laboratory location, nor should they be marked as destroyed. As such these items are to be stored to "Stored in primary packaging" enter in the Storage Box ID field.



## 6.17 Examination and sampling of manual reference samples

All manual reference samples (e.g. hair, swabs, Guthrie cards, fingernails etc.) are to be examined by the Evidence Recovery team and must be registered as "Reference" in the forensic category field to ensure that the sample is allocated to the correct Analytical batch. Inform the Quality and Projects team if this is not the case upon examination as the category will need to be changed. A notation will be added to the exhibit to state the reason for the change "Category changed to "Reference" to ensure correct reference processing". All samples that are designated as reference samples **must** be examined and sampled on Examination **Bench 15** (FDNA-EREB-0015).

Manual reference samples will be tracked to a storage box labelled "Evidence Sample – Manual" by the Quality and Projects team and placed on the daily shelves in the exhibit room (FDNA-EXSH-0696 – FDNA-EXSH-0700). The sample will be visible on the Examination worklist.

The principles of examination and sampling of reference samples are the same as those for casework exhibits.

- 1. Click the create exhibit test icon in the exhibit analytical/testing table and select Item Exam from the dropdown menu in the process field.
- 2. In the Notes field, write a brief description of the sampling performed.
- 3. Click on the save button.
- 4. Click the create exhibit test icon in the exhibit analytical/testing table and select Subsample from the dropdown menu in the process field.
- 5. In the SubID field click the plus icon in the forensic exhibit no field to auto assign enter a new barcode; this barcode will go onto the sample tube. A printed barcode must also be attached to the outside of the reference packaging i.e. envelope, CSPB etc. for tracking purposes.
- In the SubType dropdown menu select EREF.
- Examine the reference sample appropriately. For FTA cards excise ~5mm x ~5mm
  section from each of the black circles on the FTA card and place into an appropriately
  labelled 2mL tube.
- 8. In the Storage Box ID field scan the barcode of the ERT-AS box.
- Scan the barcode that is affixed to the bag of tubes in the Tube Lot No field.
- 10. Select DNA Extraction from the Technique field dropdown menu.
- 11. Select Maxwell 16 DNA IQ from the Method field dropdown menu and press the save button.



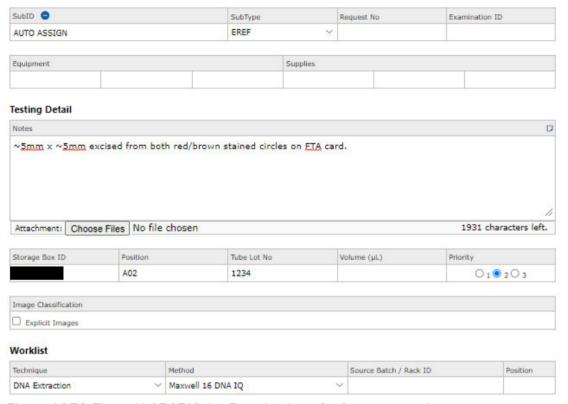


Figure { SEQ Figure \\* ARABIC } – Examination of reference samples

# 7 Associated Documentation

QIS: 14576 - Blood and Body Fluid Biological Exposure

QIS: <u>17185</u> – Detection of Azoospermic Semen in Casework Samples

QIS: 17186 - The Acid Phosphatase screening test for seminal stains

QIS: 17189 - Examination For & Of Spermatozoa

QIS: 17190 - Tetramethylbenzidine Screening Test for Blood

QIS: <u>22857</u> – Anti-contamination Procedure

QIS: 23849 - Common Forensic DNA Analysis Terms and Acronyms

QIS: <u>23959</u> – Storage Guidelines for Forensic DNA Analysis

QIS: 30800 - Investigating Adverse Events in Forensic DNA Analysis

QIS: <u>33771</u> – Examination of in-tube samples

QIS: 33798 - Examination of Sexual Cases

QIS: 33998 - Phadebas Test for Saliva

QIS: 34300 - Examination of post mortem and associated samples from deceased persons

#### 8 References

AS2243.1:2005 Safety in Laboratories Part 1 - General

Workplace Health and Safety Act 2011

Workplace Health and Safety Regulation 2011

Workplace Health and Safety Advisory Standards - various

Health, safety and wellbeing I HSQ staff site



# 9 Amendment History

Version	Date	Updated By	Amendments
1	10/06/2016	A Houlding	First issue.
2	14/06/2017	A Ryan	Added storage procedure, reference sample examination, explanation of subsamples and destruction of packaging. Added subsample label printing. Added annotating images. Moved all the general principles for sample selection, sampling techniques and specific examination strategies to the beginning of the document. Added entering exhibit result lines. Added workflow appendices
3	12/12/2017	A McNevin	Minor edits to reflect FR updates/enhancements and procedure changes, inclusion of pooling, return to primary packaging and multiples items in one package, inclusion of contents of archived SOP 17135.
4	02/11/2018	N Roselt	Minor edits to reflect current FR processes/enhancements. Updated screenshots to reflect FR enhancements. Added information to description of exhibits section (6.6). Updated process for lubricant testing. Added information regarding invasive sampling techniques. Updated pooling appendix and reference sample workflow. Inclusion of workflow for submitting retained portions of cig butts (appendix 6). Added standardised wording for request/tasks
5	05/09/2019	S Byrne	Changes to reflect new equipment and usage for examination of syringes, needles and similar sharps – safety and procedure. Amend typo's and some wording, add exhibit testing procedure with changes: Worklist and Method now added at validation.
6	30/07/2021	K Morton A McNevin	Added new standard wording for request/tasks. Added procedure for handling large fluid samples. Updated referenced and associated documents. Updated and added INT result line to appendix. Added new hair process for items and removed hair associated document. Removed lubricant testing procedure. Updated pooling process. Added additional information on syringes Removed

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Version	Date	Updated By	Amendments	
			Forensic Register from document title, new template.	
7	26/04/2022	K Morton	New template, updated screenshots and content to reflect current procedures. Added exhibit test incorrection process. Removed requirement for notation in pooled samples. Amended appendices titles.	

# 10 Appendices

- 1 Appendix 1: Exhibit Result Lines
- 2 Appendix 2: Workflow for basic item submitted in entirety
- 3 Appendix 3: Workflow for basic item partial submission (rest of item returned)
- 4 Appendix 4: Workflow for basic item with retained portion
- 5 Appendix 5: Workflow for multiple items in one CSSE
- 6 Appendix 6: Workflow for submitting retained portions
- 7 Appendix 7: Pooling of samples
- 8 Appendix 8: Standardised wording for request/tasks
- 9 Appendix 9: Incorrect exhibit tests

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# 10.1 Appendix 1: Exhibit Result Lines

Table 1 - Exhibit result lines

Exhibit Result Line	Automatic or Manual	Functions that trigger automatic results
1BPPSR – Presumptive blood test pos. Submitted-results pending	Automatic	TMB pos box ticked <b>and</b> DNA extraction selected
HAIRNFA – Hair located – not examined at this time	Manual	N/A
HOIS – Hair located on the outside of an in-tube submission	Automatic	"Hair located on the outside of tube" box ticked
IPNE – Items Prioritised. Not examined at this time	Manual	N/A
ISCB – Incorrect submission of cigarette butt	Manual	N/A
LDIS – Labelling discrepancy	Automatic	"Labelling discrepancy" box ticked
MIES – Sample required manual intervention - excess substrate	Automatic	"Excess substrate" box ticked
MIISB – Multiple items incorrectly submitted under single barcode	Manual	N/A
MISSTL – Sample required manual intervention - swab stick too long	Automatic	"Swab stick too long" box ticked
MITRI – Sample reqd manual intervention- tlift rolled incorrectly	Automatic	"Tapelift rolled incorrectly" box ticked
MNS – Micro neg for sperm	Automatic	See appendices 1 & 2 in QIS 33798 Examination of Sexual Cases
NBOS – No barcode on sample	Automatic	"No barcode on sample" box ticked
PAPPRP – Presump. PSA test positive, submitted - results pending	Automatic	See appendices 1 & 2 in QIS 33798 Examination of Sexual Cases
PBNSC – Presumptive blood test neg. Submitted for cells	Automatic	TMB neg box ticked and DNA extraction selected
PBTN – Presumptive blood test negative	Automatic	TMB neg box ticked (no extraction method selected)
PPSRP – Presump. AP test positive, submitted - results pending	Automatic	See appendix 2 in QIS 33798 Examination of Sexual Cases
PREBT – Presumptive blood test positive	Automatic	TMB pos box ticked (no extraction method selected)
PSNSC – Presump saliva negative. Submitted for cells	Automatic	Phadebas neg box ticked and DNA extraction selected
PSPSRP – Presump saliva positive. Submitted-results pending	Automatic	Phadebas pos box ticked and DNA extraction selection.
PSTN – Presump saliva test negative	Automatic	Phadebas neg box ticked (no extraction method selected)
PSTP – Presump saliva test positive	Automatic	Phadebas pos box ticked (no extraction method selected)
SEMND – Semen not detected	Manual	N/A
SOHAA – Sample on hold, awaiting advice	Manual	N/A
SPPDNA – Micro positive for sperm. Submitted-results pending	Automatic & Manual	See appendices 1 & 2 in QIS 33798 Examination of Sexual Cases
SRMI – Sample required manual intervention prior to DNA extraction	Automatic	"Other manual intervention required" box ticked

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Exhibit Result Line	Automatic or Manual	Functions that trigger automatic results
SRP – Submitted-results pending	Automatic for in-tubes Manual for items	In-tube process selected and "DNA Extraction" selected in the technique field
TRQ – Testing restarted on advice from QPS	Manual	N/A
EXREV – Extra information on reverse of crime scene sample envelope	Automatic	"Additional Information on reverse of CSSE" box ticked
NWQPS – No further work required as per advice from QPS	Automatic / Manual	"No Testing Required" box is ticked by QPS Result can be added manually if advised appropriately
INT – Item has been examined/sub-sampled	Manual	N/A
PSFTN – Presump seminal fluid test negative	Manual	See appendix 2 in QIS 33798 Examination of Sexual Cases

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# 10.2 Appendix 2: Workflow for basic item submitted in entirety

- 1. Scan barcode into the key identifier search icon, press enter or click search.
- 2. Using the exhibit movement table, track item to an examination bench.
- 3. Click thumbnail of CSSE. Check image. Close the image window.
- 4. Add additional images: Upload images into the Item Exam.
- 5. Check testing requirements for biological screening.
- Exhibit Analytical/Testing table → create exhibit test 
   icon
  - In the process field select Item Exam
  - Tick the relevant boxes under packaging and sample assessment notes
  - Use the notes field to enter examination notes, use the tick boxes if required
  - Enter a storage box barcode
  - Enter a tube lot number barcode
  - Save
- 7. Exhibit Analytical/Testing table → create exhibit test icon
  - · In the process field select result
  - Select the appropriate result from the dropdown menu under Police Report
  - Save
- 8. Put CSSE in destruction box.



# 10.3 Appendix 3: Workflow for basic item partial submission (rest of item returned)

- 1. Scan barcode into the key identifier search icon, press enter or click search.
- 2. Using the exhibit movement table, track item to an examination bench.
- 3. Click thumbnail of CSSE. Check image. Close the image window.
- 4. Add additional images: Upload images to the Item Exam.
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam
  - Tick the relevant boxes under packaging and sample assessment notes
  - In the notes field type examination notes e.g. description of item and the sampling strategy
  - Save
- 6. Create subsample for portion that is being submitted.
  - Exhibit Analytical/Testing table → create exhibit test 

    icon
  - In the process field select Subsample
  - In the SubID click the plus icon in the forensic exhibit no field to auto assign a new barcode
  - In the SubType dropdown list select MISC
  - In the notes field add a description of the subsample
  - Save
- 7. Convert sample to child exhibit.
  - Click on Exhibits tab
  - Click the add button
  - Enter barcode of subsample that was just created in the Forensic Exhibit No field
  - In the forensic category field select the relevant subsample type
  - Add description of subsample
  - In the Located/Owner field copy the relevant description from the parent item. Include any ownership details from the parent item into the "Located/Owner" field
  - Enter the parent barcode into the Parent Barcode field
  - Tick the following boxes: "Admission/Intel" and "Sample has been collected in strict compliance with CSE101 Biological Evidence"
  - Add a FR User ID in the Delivery Officer Rego field; press tab for surname to auto fill. Select Queensland Health Scientific
  - Save
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam (brief description of what the item is)
  - Add a storage location (ERT-AS storage box) and tube lot number
  - Save
- Exhibit Analytical/Testing table → create exhibit test icon
  - . In the process field select Result
  - Select the appropriate result from the dropdown menu labelled Police Report
  - Save
- 10. Create new exhibit movement for the parent barcode and track to a returns location.



# 10.4 Appendix 4: Workflow for basic item with retained portion

- 1. Scan barcode into the key identifier search icon, press enter or click search.
- 2. Using the exhibit movement table, track item to an examination bench.
- 3. Click thumbnail of CSSE. Check image. Close the image window.
- 4. Add additional images: Upload images to the Item Exam.
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam
  - Tick the relevant boxes under packaging and sample assessment notes
  - Tick relevant boxes in the testing detail tables
  - Add a storage location (ERT-AS box)
  - Add tube lot number
  - Save
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Subsample
  - In the SubID field click the plus icon in the forensic exhibit no field to auto assign a new barcode
  - In the SubType field click RETAIN
  - · Fill in details of what was retained in the notes field
  - Add an item retention storage box barcode
  - Add a tube lot number
  - Save
- 7. Exhibit Analytical/Testing table → create exhibit test icon
  - . In the process field select Result
  - Select the appropriate result from the dropdown menu labelled police report
  - Save
- 8. Put CSSE into the items destruction box



# 10.5 Appendix 5: Workflow for multiple items in one CSSE

- 1. Scan barcode into the key identifier search icon, press enter or click search.
- 2. Using the exhibit movement table, track item to an examination bench.
- 3. Click thumbnail of CSSE. Check image. Close the image window.
- 4. Add additional images: Upload images to the Item Exam.
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam
  - Tick the relevant boxes under packaging and sample assessment notes
  - Type relevant information on what is contained within the CSSE into the notes field
  - Save
- Create subsamples (or an Examination Record) for individual items. Exhibit Analytical/Testing table → create exhibit test icon
  - · In the process field select Subsample
  - In the SubID field click the plus icon in the forensic exhibit no field to auto assign a new barcode
  - In the SubType dropdown list select MISC
  - In the notes field add a description of the subsample
  - Save
  - Repeat for subsequent items.
- 7. Convert subsamples to child exhibits
  - Click on exhibits tab
  - Click the add button
  - Enter barcode of subsample that was just created in the forensic exhibit no field
  - In the forensic category field select the relevant subsample type
  - Add description of the subsample
  - In the Located/Owner field copy the relevant description from the parent item.
     Include any ownership details from the parent item into the "Located/Owner" field
  - In the parent barcode field add the parent barcode
  - Tick the following boxes: "Admission/Intel" and "Sample has been collected in strict compliance with CSE101 Biological Evidence"
  - Add a FR User ID in the Delivery Officer Rego field; press tab and your surname will automatically appear. Select Queensland Health Scientific
  - Save
  - Click the back button and repeat for subsequent subsamples
- 8. Complete an item exam the first child exhibit. Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam
  - Tick relevant boxes under cigarette butt notes and fill in details in notes field.
  - Add a storage location (ERT-AS box)
  - Add tube lot number
  - Save



- 9. Exhibit Analytical/Testing table → create exhibit test create exhibit test
  - In the process field select Subsample
  - In the SubID field click the plus icon in the forensic exhibit no field to auto assign a new barcode
  - In the SubType field click RETAIN
  - Fill in details of what was retained in the notes field
  - Add an item retention storage box barcode
  - Add a tube lot number
  - Save
- 10. Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Result
  - Select the appropriate result from the dropdown menu labelled police report
  - Save
- 11. Repeat steps 7 9 for subsequent items
- 12. Put CSSE into the items destruction box
- 13. Add new exhibit movement for the parent item and select DESTROYED in the location field
- 14. In parent Item Exam Exhibit Analytical/Testing table → create exhibit test con
  - · In the process field select Result
  - Select: MIISB Multiple items incorrectly submitted under single barcode
  - Save



# 10.6 Appendix 6: Workflow for submitting retained portions

- 1. Scan barcode into the key identifier search icon, press enter or click search.
- 2. Click subsample time/date hyperlink and use the subsample movement table to track item to an examination bench.
- Exhibit Analytical/Testing table → create exhibit test icon
  - In the process field select Item Exam
  - In the notes field type examination notes e.g. description of retained portion and the sampling strategy
  - Save
- Create a subsample (if necessary) for any remaining portions of the retained subsample to be submitted. Exhibit Analytical/Testing table → create exhibit test icon
  - · In the process field select Subsample
  - In the SubID field click the plus icon in the forensic exhibit no field to auto assign a new barcode
  - In the SubType dropdown list select MISC
  - In the notes field add a description of the subsample
  - Save
  - Repeat for subsequent portions (if necessary).
- 5. Convert original subsample barcode to child exhibit
  - Click on exhibits tab
  - Click the add button
  - Enter barcode of the subsample into the forensic exhibit no field
  - In the forensic category field select the relevant subsample type (cigarette butt)
  - Add description of the subsample
  - In the Located/Owner field copy the relevant description from the parent item.
     Include any ownership details from the parent item into the "Located/Owner" field
  - In the parent barcode field add the parent barcode
  - Tick the following boxes: "Admission/Intel" and "Sample has been collected in strict compliance with CSE101 Biological Evidence"
  - Add a FR User ID in the Delivery Officer Rego field; press tab and your surname will automatically appear. Select Queensland Health Scientific
  - Save
  - · Click the back button and repeat for subsequent subsamples
- Complete an item exam on all child exhibits. Exhibit Analytical/Testing table → create
  exhibit test 
  icon
  - In the process field select Item Exam
  - In the notes field type examination notes e.g. description of retained portion
  - Add a storage location (ERT-AS box)
  - Add tube lot number (unless portion is remaining in original retention tube, this should be noted)
  - Save
- Exhibit Analytical/Testing table → create exhibit test 
   icon
  - In the process field select Result
  - Select the appropriate result from the dropdown menu labelled police report
  - Save



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## 10.7 Appendix 7: Pooling of samples

- Complete an item exam on the parent barcode, create a RETAIN subsample for any retained portions (e.g. cigarette filter) and create an appropriate number of MISC subsamples.
- 2. Convert MISC subsamples to child exhibits and complete an item exam for each (include the storage location and tube lot number).
- 3. Create an Analytical note against each child exhibit stating, "Hold after EXT: sample to be pooled".



Figure 10 – Exhibit Analytical/Testing and Examination tables for pooled samples

- 4. Create a new Examination Record under one of the child exhibits, enter the new child exhibit barcodes that are to be pooled together into the Exhibit/s Examined field and add "For Pooling" in the Examination Notes. All other mandatory fields are to be completed as per a regular examination record.
- Save the Examination Record and ensure the Exhibits Examined field appears as per Figure 28. The examination record will now appear in the Examinations table for each child exhibit.



Figure 11 - Exhibits Examined

- 6. Click the arrow icon next to the edit button and select add related exhibit to the examination record, click the plus icon to auto assign a new barcode (this will be the pooled barcode).
- Select the appropriate category according to what the parent item is. The Description and Located/Owner fields are auto populated. The remaining fields are to be completed as per usual processes.

**Note:** No parent barcode is required.



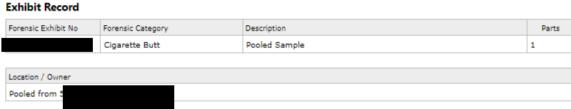


Figure { SEQ Figure \\* ARABIC } - Registration of pooled sample

The sample will now appear on the POOLING review list for analytical to check and validate.

**Note:** ER staff are **not** to validate the Pooling line on the pooled barcode as it triggers downstream processing actions in Analytical (see Figure 30).

- 9. Add an analytical note to the pooled barcode "Please add to Quant worklist".
- 10. It is not necessary to add any result lines to a pooled sample as automatic result lines are sent upon validation.

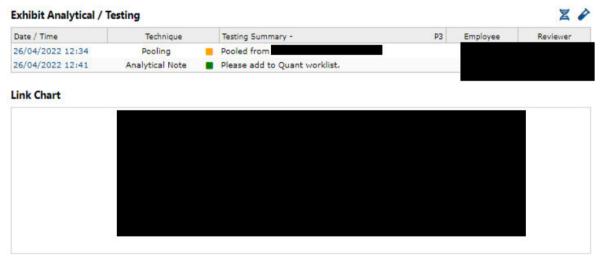


Figure 12 - Pooled sample on link chart

11. Use the Exhibit Movement table to track the parent barcode to 'DESTROYED'.

### 10.8 Appendix 8: Standardised wording for request/tasks

As each case / exhibit circumstance may be different, the wording below may be adjusted for content where appropriate.

## 10.8.1 Male/Suspect clothing for semen:

Date/Initials - Hello.

Regarding exhibit XXXXXXXXX - AP positive fabric, the FR indicates that this is a sample of fabric from underwear belonging to the suspect and semen testing is required. For cases of alleged male on female sexual assault, Semen testing on male suspect underwear is not routinely performed given that the presence of semen is not an unexpected finding. Please confirm if semen testing is still required. The item has been placed on hold pending your response. regards

Your NAME

## 10.8.2 Adult Female undies for saliva testing:

Date/Initials - Hello,

Regarding exhibit XXXXXXXX - underwear from complainant, this whole item/fabric has been submitted for saliva and seminal fluid testing. Saliva testing of the crotch area of adult female underwear is not routinely performed due to the high concentration of amylase present in vaginal secretions and faecal matter. False positive reactions, therefore, are likely. Please confirm if saliva testing is still required. The item has been placed on hold pending your response.

Your NAME

### 10.8.3 SAIK without medical notes or QP127

Date/Initials - Hello,

We have received a SAIK barcode XXXXXXXXX without any accompanying Medical notes or a QP127. These notes assist in determining how these exhibits are examined. Please confirm if notes were taken during the SAIK examination and if so please forward to Forensic DNA Analysis. The SAIK has been placed on hold pending your response. regards

Your NAME

## 10.8.4 Spelling of names on a SAIK doesn't match – paperwork/SAIK packaging/FR

Date/Initials - Hello,

We have received a SAIK barcode XXXXXXXXX which has discrepancies in the spelling of the complainants' name. The FR states XXXXXXXXX, the FMO notes state XXXXXXXXX and the SAIK packaging states XXXXXXXXXX. Please confirm the correct name of the complainant. regards

Your NAME

## 10.8.5 Lubricant testing – is it required?

Date/Initials - Hello.

We have received a SAIK barcode XXXXXXXXX into Forensic DNA Analysis with medical paperwork that states lubricant (namely XXXXXXXXX) was used during the alleged sexual assault however; the box for FSS Chemical Analysis has not been ticked. Can you please advise whether lubricant testing is required and if so, can you please tick the FSS Chemical Analysis box in the Examination Section on the Exhibit Record page. Please be aware that if lubricant testing is required a sample of the lubricant used during the alleged sexual assault will need to be sought for comparison. This SAIK has been placed on hold pending your response.

Your NAME

Queensland Government

### 10.8.6 AP Blotting paper and AP fabric received at same time

(note – if AP fabric has already been tested and is negative, do not use this wording)
Date/Initials - Hello.

We have received both an area of AP positive fabric (exhibit XXXXXXXXX) & the associated blotting paper (exhibit XXXXXXXXX) of the positive AP reaction of the same area. In our experience, the blotting paper used to perform AP testing does not yield informative results, and when AP testing with blotting paper is performed in our laboratory it is not retained for future testing. It is recommended that only the positive area(s) of the item tested be submitted for further testing. We are seeking permission to place the blotting paper (exhibit XXXXXXXXX) on-hold pending the outcome of DNA testing on the associated area of fabric (exhibit XXXXXXXXXX). If informative DNA results are obtained from the fabric, we request that the blotting paper be returned untested.

regards

Your NAME

## 10.8.7 AP Blotting paper received and AP fabric already positive result

Date/Initials - Hello.

We have received both an area of AP positive fabric (exhibit XXXXXXXXX) & the associated blotting paper (exhibit XXXXXXXXX) of the positive AP reaction of the same area. In our experience, the blotting paper used to perform AP testing does not yield informative results, and when AP testing with blotting paper is performed in our laboratory it is not retained for future testing. It is recommended that only the positive area(s) of the item tested be submitted for further testing. As results have already been obtained for the associated area of fabric (exhibit XXXXXXXXX), we are seeking permission to return the blotting paper (exhibit XXXXXXXXXX) untested. If this is acceptable, please mark the item with "No Testing Required"; alternatively, if testing is still required, please advise.

regards

Your NAME

## 10.8.8 Discrepancy for exhibit record description (Item placed on-hold):

Date/Initials - Hello,

Regarding exhibit barcode XXXXXXXXX, the Exhibit Record Description in the Forensic Register states "A B C", however the Crime Scene Envelope states "X Y Z". Please confirm the correct description for this exhibit and adjust any Forensic Register records as necessary, or alternatively the item can be returned for your correction. This item has been placed on hold pending advice. regards

Your NAME

## 10.8.9 FR number discrepancy (Item placed on-hold):

Date/Initials - Hello.

Regarding exhibit barcode XXXXXXXXX, the exhibit is registered in the Forensic Register under FR number "1 2 3", however the Crime Scene Envelope states "3 2 1". Please confirm the correct FR number for this exhibit and adjust any Forensic Register records as necessary, or alternatively the item can be returned for your correction. This item has been placed on hold pending advice. regards

Your NAME

## 10.8.10 Discrepancy for exhibit record description (Item NOT placed on-hold):

Date/Initials – Hello,

Regarding exhibit barcode XXXXXXXXX, the Exhibit Record Description in the Forensic Register states "A B C", however the Crime Scene Envelope states "X Y Z". Please confirm the correct description for this exhibit and adjust any Forensic Register records as necessary.





regards Your NAME

## 10.8.11 Discrepancy in forensic category (Item placed on-hold):

Date/Initials - Hello.

Regarding exhibit barcode XXXXXXXXX, the Exhibit Record Forensic Category in the Forensic Register states "A B C", however the exhibit received with the Crime Scene Envelope is an "X Y Z". Please confirm the correct exhibit type and adjust any Forensic Register records as necessary, or alternatively the item can be returned for your correction. The item has been placed on hold pending advice.

regards

Your NAME

## 10.8.12 Sample requires additional analysis confirmation (Item placed on-hold):

Date/Initials - Hello,

Regarding exhibit barcode XXXXXXXXX, this item has been ticked as "Sample requires additional analysis (lubricant, fibre, glass, soil etc.)" with no additional information provided as to what form of additional analysis is required. Please advise the nature of the additional analysis required, or alternatively, if none is required, please uncheck. The item has been placed on hold pending advice.

regards

Your NAME

## 10.8.13 Is semen or saliva testing required (Item placed on-hold):

Date/Initials - Hello,

Regarding exhibit barcode XXXXXXXXX. Due to the nature of this case could you please confirm whether semen or saliva testing is required. This item has been placed on hold pending advice. regards

Your NAME

## 10.8.14 Name missing from the Exhibit Description or Located/Owner fields (SAIK)

Date/Initials - Hello,

The Exhibit Record page for SAIK exhibit barcode XXXXXXXXX has no name listed in either the Exhibit Description or Location/Owner fields. This information is used (in conjunction with the exhibit barcode) as a second identifier when checking exhibit details. Can this information please be added to the registration of this exhibit so that testing may proceed. regards

Your NAME

## 10.8.15 Clothing located within SAIK

Date/Initials - Hello.

SAIK exhibit barcode XXXXXXXXX has been received. Upon opening, SAIK contains 1 x pair of socks and 1 x pair of underwear. The clothing will be repackaged in the SAIK and returned for examination by QPS Scientific. Alternatively, they can be examined by Forensic DNA Analysis, however whole item authorisation must be sought from an Inspector with details of testing requirements.

regards

Your NAME



## 10.9 Appendix 9: Incorrect exhibit tests

If there is an error in the exhibit analytical/testing table, the line which contains the error must be marked as incorrect by the user who made the error, examples of this include

- An incorrect exhibit result line has been selected
- An incorrect examination has been performed
- The examination is duplicated

A line within the exhibit analytical/testing table can only be marked as incorrect by the examiner if it has not yet been validated. If the line has been validated, or it is a line that auto-validates, it must be marked as incorrect by a Senior Scientist. **Note:** If the line has not yet been validated then the examiner should delete all information within the record before marking it as incorrect.

 Click on the date/time hyperlink of the incorrect/duplicate process in the exhibit analytical/testing table.



Figure { SEQ Figure \\* ARABIC } - Date/time hyperlink

2. Click the arrow icon next to the edit button and select "Incorrect Test.



Figure { SEQ Figure \\* ARABIC } - Incorrect Test

3. Click the [CLICK TO INCORRECT] bar.



Figure { SEQ Figure \\* ARABIC } - Click to incorrect bar

This process will now have a line through it in the exhibit analytical/testing table. As the
result was not validated before being marked as incorrect, the orange traffic light will
remain.



Figure { SEQ Figure \\* ARABIC } - Incorrect exhibit test



## Queensland Health

# Forensic and Scientific Services



# **Examination for and of Spermatozoa**

#### 1 **Purpose**

The presence of spermatozoa is a confirmatory test for the presence of semen. This document describes the method by which a scientist performs microscopic examination for the presence of spermatozoa which includes the preparation of microscopic slides from exhibits, staining of slides and interpretation of the microscopic smears/slides for spermatozoa and other cellular material.

#### 2 Scope

This Standard Operating Procedure (SOP) applies to all scientists performing the examination of items for the presence of semen.

#### 3 **Definitions**

In this document, where reference is made to spermatozoa, it refers to human spermatozoa unless otherwise specified.

#### **Principle** 4

The investigation of sexual assault cases may require the testing of exhibits collected as part of a forensic medical examination or scene examination for the presence of semen. Within the laboratory the detection of spermatozoa confirms the presence of semen. A reliable and accurate staining method is essential to aid the examining scientist the ability to differentiate between cellular types; most significantly spermatozoa from epithelial, yeast and white blood cells.

Currently the Haematoxylin and Eosin (H&E) stain is adopted for this process. The H&E staining method has been used for this purpose within the laboratory for many years. The haematoxylin (basic stain) stains the deoxyribonucleic-acid (DNA)/histone rich base of the spermatozoa head deep purplish-blue. The eosin (acidic stain) stains the acrosomal cap pink and the tail pink if the spermatozoa are intact (N.B. because Forensic DNA Analysis uses a water based eosin stain, the acrosomal cap often appears very light pink or clear). The use of counterstaining differentiates spermatozoa from most cellular debris.

Confusion with yeasts, especially monilia, can occur and extreme care must be taken when monilial infections such as thrush are suspected. With experience, spermatozoa and yeasts can be distinguished by size and/or the presence of cell walls. In addition, yeasts do not display the typical biphasic staining that spermatozoa do (refer staining characteristics in paragraph above).

Haematoxylin is a natural dye. Its active colouring agent is haematin, which is formed by the oxidation of haematoxylin. This oxidation process or "ripening" occurs when

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haematoxylin solutions are allowed to stand for several days. However, the process can be accelerated with the introduction of an oxidising agent such as sodium iodate. During oxidation the haematoxylin loses two atoms of hydrogen, and its formula changes from  $C_{16}H_{14}O_6$  to  $C_{16}H_{12}O_6$ . Sufficient haematoxylin should be left unoxidized in the solution so that natural oxidation can continue thus prolonging the shelf life and useability of the stain. Completely oxidized haematoxylin becomes colourless. As the oxidation process occurs when haematoxylin is exposed to light and continues over the life of the solution, haematoxylin should be stored in dark bottles until ready for use. Haematoxylin is an excellent nuclear stain. Haematin, via the aluminium ion mordant, binds to the anionic sites in the nuclei (a mordant is a substance that causes certain staining reactions to take place by forming a link between the tissue and the stain). At this stage the nuclei stain red, which is then converted to the blue-black colour when the pH is raised (by a weak alkali wash such as Scott's tap water substitute in some H&E staining methods) known as "blueing".

To avoid stain precipitation on the slide, the haematoxylin solution must be filtered. It should be changed immediately if staining quality deteriorates.

Eosin is an acid dye which combines electrostatically with the acidophilic tissue components such as cytoplasm (an anionic dye that stains the cationic tissue components). Alcoholic and acidified solutions of eosin tend to stain much more vividly than do the aqueous solutions. With water soluble eosin, rinse in water very quickly or else eosin will wash out.

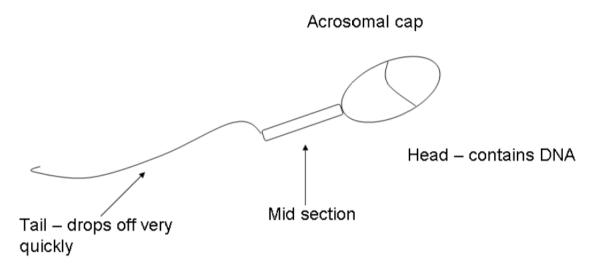


Figure { SEQ Figure \\* ARABIC } Spermatozoa

## 5 Actions – Staining procedure

## 5.1 Slide Staining

Slides are created by Analytical Scientists and stored to a slide box, this box is tracked to the Analytical laboratory. The slide box containing the stored and prepared slides are retrieved by Evidence Recovery staff from the extraction sorting hatch and tracked to the Evidence Recovery laboratory. Individual slides are then tracked to the Evidence Recovery laboratory and the empty slide box is returned to the extraction sorting hatch after processing.

Add a Microscopic process from the Exhibit testing table, complete the SubID, SubType and the Reagents lot numbers fields in the Forensic Register.



## Testing / Analysis

440131 CALDWELL, V PSD FSS

Date	Process	SubID	SubType	Equipment No
31/01/2022 15:25	Microscopic		SLIDE	

Results of Microscopy Examination			
O No spermatozoa were detected.	No result.		
	N2)		

Reagents			
22987-707942	24926-714428		
Haematoxylin Harris non toxic	Eosin Y 1% Aqueouus		

Notes	
Whole Sperm: Sperm Heads: Epithelial Cells: Other: Magnification: x400	
Attachment:	

Figure { SEQ Figure \\* ARABIC } Creating a microscopic process

Microscopic slides are stained using Haematoxylin and Eosin (H&E). The method for performing manual staining is as per procedure detailed in Appendix 1.

## 5.2 Microscopic Examination

Examine slide using the x40, x50 or x100 objectives. Quantitate the number of whole spermatozoa, spermatozoa heads and epithelial cells observed in the "Microscopic" process under subsample barcode of the microscopy slide. Do this by appending the preformed text using the following criteria as a guide:

Table 1 Quantitation criteria

Quantity	Description
0	None seen
<1+	Very hard to find *
1+	Hard to find
2+	Easy to find
3+	Very easy to find
4+	Abundant

<sup>\*</sup> If less than ten spermatozoa are observed on the whole slide, a quantitation of <1+ must be used and for at least one spermatozoa, note the location on the slide with the use of the England Finder Graticule (see Appendix 3).

Human spermatozoa are distinguished from non-human mammalian sources by their morphology and by their behaviour toward H&E, resulting in a purplish/blue head and light pink/clear cap (see Section 5.4). Record whether there are bacteria or yeast present next to "Other:" If no bacteria or yeast seen record "N/A".

The default Magnification: x400 is used when adding the Microscopic process. Edit if applicable, e.g. x500. Record the Equipment No. used and select the appropriate radio button in the Results of Microscopy Examination field.



## Testing / Analysis

Process**	Date	SubID	SubType		Equipment No	
Microscopic ~	01/12/2021 08:27		SLIDE V		200420451	
	Res	sults of Microscopy Exa	amination			
O Spermatozoa were	detected.	O No spermatozoa	were detected.		No result.	
		Reagents				
21395-20200812						
Notes						
Whole Sperm: Sperm Heads: Epithelial Cells: Other:						

Figure { SEQ Figure \\* ARABIC } Recording microscopy findings in Microscopic Process

## 5.3 Animal Semen

Animal spermatozoa are morphologically different to human spermatozoa and react differently to staining. Where suspected spermatozoa are located which are morphologically different to human spermatozoa, the examining scientist should consider the possible presence of animal spermatozoa. N.B. Forensic DNA Analysis does not identify or characterise animal spermatozoa.

## 5.4 Spermatozoa Interpretation

Magnification: x500

Attachment: Choose File No file chosen

If slides are stained properly spermatozoa should be easily distinguished from epithelial cells, cellular debris, fibres etc. Spermatozoa heads can look similar in shape and colour to yeasts, however they do have different staining characteristics. If in any doubt consult an experienced examiner.

The recovery of semen is dependent on a number of factors but not limited to

- The amount of spermatozoa in the ejaculate
- The amount of ejaculate
- The environment the ejaculate is deposited on
- Washing
- Douching
- Menstruation
- Efficiency of the sampling process
- Time between ejaculation and sampling
- Storage of the samples
- Natural drainage or degradation of spermatozoa in certain environments

With respect to the above influences, the time since ejaculation has occurred can only be estimated. A number of studies have been conducted regarding the persistence of spermatozoa in the vagina. References to these studies can be found in Appendix 2.

### 6 Records

Nil

## 7 Quality assurance/acceptance criteria

Controls are used to test the quality and validity of the staining reagents prior to use. A positive control slide should be tested and read by a Scientist prior to the staining of slides for microscopy, (once daily), each time a new batch of Haematoxylin and Eosin solution is received/opened and when positive control slides are prepared.

### 7.1 Creation of H&E control slides

Collect human semen in a sterile yellow-capped Specimen jar. The tube is to be labelled with the following information:

- Sperm donor number
- Date and time of collection

Each new collection of positive control material should be checked with a previously accepted batch of stain.

The semen is to be stored within a freezer until required to create H&E positive control slides using the following process:

- 1. Clean heating block using bleach and 70% ethanol solution.
- Clean frosted microscope slides with ethanol and label with white label (H&E Pos Ctrl: Sperm donor number; Lot No.).
- 3. Spread slides out on heating block to heat before use.
- 4. Clean automatic pipette with bleach and 70% ethanol solution.
- 5. Using a new filtered pipette tip, add 20µL of the neat semen to 10.0ml nanopure water using a clean 10ml tube. Vortex.
- 6. Add 20 μL of the diluted semen solution to each slide, put a circle around the sample using a black marker pen.
- 7. Heat fix the slides on a heating block at 50°C for approximately 30 minutes.
- 8. Store the slides in labelled plastic slide box "Unstained H&E Positive Control Slide Storage" and store the box in Rm 6124.

## 7.2 Testing and interpretation of control slides

The following process is used to test, interpret and record control slide results:

- 1. Remove a H&E control slide from slide box, label with date, initials, and stain with H&E using the method in Appendix 1.
- 2. Dry slide on heating block at 50°C.
- 3. Coverslip slide using Pertex® mounting medium. A small amount of xylene can be used to assist with slide mounting.
- 4. A scientist must examine and pass or fail control slides microscopically before processing exhibit slides can occur.
- Completed control slides get transferred to a plastic box labelled H&E Control Slide Storage box #
- Once a slide box is full of completed positive control slides, write the date range of the slides contained within the box on the outside of the box and transfer the box to Rm 6106B, 'Exhibit Room' for long term storage.



Acceptance of the reagents is based on the interpretation of the Positive control slide. The following criteria must be met before passing the reagent for use:

- 1. Spermatozoa head stains a deep purplish-blue.
- 2. Acrosomal cap stains light pink/clear.
- 3. Tail stains pink.

In the event the control slide fails the following process is to be completed:

- 1. Repeat the staining procedure with a new control slide and assess as above.
- 2. If the control slide fails again then discard the in-use stain and then stain a new slide using a fresh batch from the stock solutions and assess slide as above.

### 8 Associated Documentation

QIS: 17185	Detection of Azoospermic Semen in Casework Samples
QIS: <u>17186</u>	The Acid Phosphatase screening test for seminal stains
QIS: 23849	Common Forensic DNA Analysis Terms and Acronyms
QIS: <u>25747</u>	Use and routine care of compound optical and stereo microscopes
QIS: 30800	Investigating Adverse Events in Forensic DNA Analysis
QIS: 33798	Examination of Sexual Cases
QIS: 33800	Examination of Items
QIS: 34103	Receipt, Storage and Preparation of Chemicals, Reagents and Kits in
	Forensic Register

## 9 References

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  Example
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#### 10 **Amendment History**

Revision	Date	Author/s	Amendments
0	Unknown	Unknown	Unknown
1	Unknown	Unknown	Unknown
2	Unknown	Unknown	Unknown
3	27 Nov 2002	V lentile	Format updated, manual staining to appendix.
			Removed notes on examination of swabs,
			removed unpublished paper, as work wasn't
			completed.
4	19 Nov 2003	L Freney	Updated references
5	12 Jul 2006	J Howes/A	"Reference" put after "Actions".
		Williamson	
6	05 Aug 2006	J Howes	Added in Sexual Assault Investigation
			Flowcharts, examination of SAIK Swabs,
			Photograph or Witness required for ++ (1+)
			sperm and PSA test.
7	23 Oct 2006	J Howes	Reporting results Eg. ++ or 2+
8	25 Jun 2007	J Howes	Unified grading scale comments. Added
			Crimelite flowchart.
Version	Date	Updated By	Amendments
9	13 Mar 2008	QIS2 Migration	Headers and Footers changed to new CaSS
		Project	format. Amended Business references from
			QHSS to FSS, QHPSS to CaSS and QHPS to
10	40 July 2040	Alleyel	Pathology Queensland
10	16 July 2010	A Lloyd	Removal of Crimelite in scope and the
			Crimelite flowchart. Changed section 2.2 to include use of suspensions. Removal of
			section 2.8 – Vaginal Secretions. Changes to
			section 2.0 – vaginal secretions, changes to section 2.10 to remove AP testing on smears
			positive to spermatozoa. Photograph or
			locations required for smear with 1 or 2 sperm
			seen. Clarification of flowchart regarding
			previously screened items by QPS. Changes
			to SAIK flowchart. Removal of animal sperm
			diagrams and insertion of photographs of
			animal sperm.
11	03 Feb 2011	A Lloyd	Amended use of vernier for slides to use of the
			England Finder Graticule.
12	31 Oct 2013	A Lloyd	Removed animal sperm photos. Amended
		J Seymour-	workflow charts, changed headings from CASS
		Murray	to HSSA. Change H&E solutions and staining,
			add England Finder information. Updated
40	00 1 1 55 15		some hyperlinks.
13	03 July 2015	J Seymour-	New template, update hyperlinks, some
		Murray	formatting updates and minor wording
44	47 5-1	A MaNteride	changes.
14	17 February	A McNevin	Added storage of cell suspensions at 4 °C,
	2017		removed "The" from title, typographical
			corrections, included product name for
15	20 August	A McNevin	mounting medium,  Additional references added; further
เอ	29 August 2018	A WICHEVITI	·
	2010		information added to Appendix 2 regarding persistence of spermatozoa in oral and anal
		<u> </u>	persistence of spermatozoa in oral and anal



			samples; other minor changes reflecting FR processes
16	06 May 2020	N Roselt, A McNevin	Minor updates
17	01 December 2021	C Chang	New Template. Added QIS:25747 hyperlink. Formatting updates. Updated document to reflect current processes.

#### 11 **Appendices**

- Appendix 1: H & E Manual Staining Procedure 1
- 2
- Appendix 2: Persistence of Spermatozoa
  Appendix 3: England Finder Package Insert



## 11.1 Appendix 1: H & E Manual Staining Procedure

### 11.1.1 Chemical Hazards

## **Pertex Mounting medium**

WARNING: Pertex is irritating to eyes, respiratory system and skin.

Wear PPE and eye protection.

## 11.1.2 Manual Staining Procedure

Staining is performed in the staining fumehood in Rm 6124, Evidence Recovery laboratory, Forensic DNA Analysis.

### Procedure:

- 1. Place slide on staining rack over sink, stain with haematoxylin for five minutes (add one volume and let rest)
- 2. Wash with nanopure water.
- 3. Stain with eosin for one minute.
- 4. Wash with nanopure water (quick wash).
- 5. Allow to dry on hot plate.
- 6. Mount coverslip in Pertex

## 11.1.3 Staining Quality Controls

The following quality steps should be implemented:

 Haematoxylin should be filtered before use as the crystals in solution can result in stain deposit on the slides which affects the reading of the slide. (Once a month) Filtering should also occur when refilling the Schott bottle from the stock solution. Eosin does not require filtering.

Commercial haematoxylin and eosin have expiry dates which are added to the reagent's registration page

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## 11.2 Appendix 2: Persistence of Spermatozoa

The following information is provided to assist with the provision of expert opinion evidence in court.

Literature provides a range of time periods for the persistence of non-motile spermatozoa in the vagina:

- Up to 24 Hours¹
- Up to 3-4 days<sup>2</sup>
- Up to 9 days or 12 days in the cervix, sometimes after menstruation<sup>3</sup>
- Up to 3 to 4 days, but may be longer<sup>4</sup>

Literature provides a range of time periods for the persistence of motile spermatozoa in the vagina:

- The number of motile spermatozoa discernible in the vagina may be normal after one hour and markedly decreased after 2 hours; after 3 hours normally no spermatozoa are found.
   Menstruation often prolongs motility in the vagina to as long as 4 hours compared with the normal period of 30 to 45 minutes.<sup>5</sup>
- Spermatozoa remain motile in the vagina for 2 to 3 hours and in the cervix for 48 to 110 hours<sup>6</sup>
- Normally 10% of the spermatozoa are alive in the vagina at the end of 2 hours post coitum.
   Variations in number and motility depend upon the pH of the vagina and semen, quantity of semen deposited, bacteria and flora of the vagina and the time examined post-coitally. The author has seen motile spermatozoa in the vaginal pool after 8 hours.<sup>7</sup>
- In several cases in which repeated examinations were possible before conception occurred, all motility ceased within one hour after intercourse. A fall of motility to 10% within 30 minutes is compatible with fecundity. On the other hand, spermatozoa may continue to move for 3 hours in a normal untreated vagina.<sup>8</sup>
- The motility of the spermatozoa in the specimen may give a clue to their length of stay as they remain motile from 30 to 60 minutes after deposition in the vagina.<sup>9</sup>

Literature provides a range of time periods for the persistence of spermatozoa in the oral cavity

- One study shows that the expectation of observing a sperm-positive oral swab is very low 15 hours post assault, with the longest time period being 27 hours, and no positives at 48 hours.<sup>10</sup>
- Other studies have shown a maximum time of 6 hours<sup>11</sup> and 24 hours (with one example of >48hours in deceased person with time since intercourse based on time of death).<sup>12</sup>



<sup>&</sup>lt;sup>1</sup> O.J. Pollack. 1963 Arch. Pathology 35 p140-184

<sup>&</sup>lt;sup>2</sup> Gordon, Turner and Price 1965 Medical Jurisprudence

<sup>&</sup>lt;sup>3</sup> Morrison 1972 Brit. J. Vener. Dis 48 p141

<sup>&</sup>lt;sup>4</sup>Gordon, Turner and Price 1965 Medical Jurisprudence

<sup>&</sup>lt;sup>5</sup> O.J. Pollack. 1963 Arch. Pathology 35 p140-184

<sup>&</sup>lt;sup>6</sup> Weisman 1941 Spermatozoa and Sterility

<sup>&</sup>lt;sup>7</sup> Wm.Heinmann Medical Books Ltd 1945 Fertility in Women

<sup>&</sup>lt;sup>8</sup> Hamish Hamilton Medical Books 1948 Sterility and Impaired Fertility

<sup>&</sup>lt;sup>9</sup> Gonzales, Vance, Helpern and Umberger 1954 Legal Medicine

<sup>10</sup> Casey et. al. 2016 Journal of Forensic Sciences p1-8

<sup>&</sup>lt;sup>11</sup> Willott & Allard 1982 Forensic Science International p135-154

<sup>&</sup>lt;sup>12</sup> Nittis et al 2016 Journal of Forensic and Legal Medicine p92-97

Literature provides a range of time periods for the persistence of spermatozoa in anal and rectal swabs.

- One study shows that spermatozoa with tails are rare to find (observed at 4 and 6 hours post intercourse) and sperm heads were observed up to 46 hours post intercourse (with one example of 65 hours).<sup>13</sup>
- Another study indicates sperm can persist for up to 48 hours post intercourse, with the longest recorded time period being 85 hours, however it is unlikely that sperm will be detected on internal swabs beyond 24 hours.<sup>14</sup>



<sup>&</sup>lt;sup>13</sup> Willott & Allard 1982 Forensic Science International p135-154

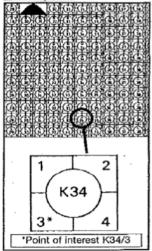
<sup>&</sup>lt;sup>14</sup> Casey et. al. 2016 Journal of Forensic Sciences p1-8

## 11.3 Appendix 3: England Finder Package Insert

## The England Finder

The England Finder is a glass slide marked over the top surface in such a way that a reference position can be deduced by direct reading, the relationship between the reference pattern and the locating edges being the same in all finders. The object of the Finder is to give the microscopist an easy method of recording the position of a particular field of Interest in a specimen mounted on a slide, so that the same position can be re-located using any other England Finder on any microscope.

### Description



The England Finder, a section of which is illustrated, consists of a glass slide 3" x 1" marked with a square grid at 1mm intervals. Each square contains a centre ring bearing reference letter and number, the remainder of the square being subdivided into four segments numbered 1 to 4. Reference numbers run horizontally 1 to 75, and letters vertically A-Z (omitting I). The main locating edge is the bottom of the slide which is used in conjunction with either the left or right vertical edge of the slide, according to the fixed stops of the stage of the microscope, all three locating edges being marked with arrow heads. The label on the finder should always appear visually at the bottom left corner when through most microscopes the reference image will appear correct.

In the illustration (part shown), the point of interest is marked with a cross, and will be seen to lie in the third segment of the square of reference K34, hence the England Reference is K34/3.

### Method of Use

- 1. Mark the specimen slide with a label on the left indicating with arrows which sides are to be used for location. Place the slide on the stage of the microscope bringing the bottom long edge in contact with the base stops of the stage and then sliding either left or right into contact with the vertical fixed stops as appropriate. It is important always to obtain the main location of the slide and finder on the base stops first.
- Having examined the specimen in the normal way and found a point of interest, bring this to the centre of the field of view (a crosswire in the eyepiece is useful in this respect).
- 3. Taking care not to alter the position of the fixed stops of the stage, remove the slide and replace with the England Finder, again bringing the bottom edge in contact first and sliding to the appropriate vertical stop, the label of the Finder being at the bottom left corner.
  - 4. The reference pattern of the Finder will now be seen through the microscope (adjusting the focus if necessary). The reference number of the main square is recorded followed by an oblique stroke and the number of the segment in which the centre of the field of view lies (1 to 4 or 0 if in the centre circle). The boundary lines of the main squares are easily distinguishable as these are the only continuous straight lines of the pattern.
  - 5. The reverse procedure is adopted to re-locate the point of interest, The England Finder is placed on the stage as outlined above and the stage is adjusted until the appropriate reference square and segment appear in the centre of the field of view. Remove the finder and replace with the specimen slide with label to left and appropriate vertical slide in contact with the fixed stop, when the point of interest will appear in the centre of the field of view.

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## 11.3.1 England Finder Graticule Use

Before use ensure stage slide holder is in correct position (slide holder should be in the correct position as it is not removed on cleaning but if not-hold with one hand push holder back to full extent against the screws, tighten screws while holding and check for correct positioning).

- 1. Place graticule on stage with labelled corner at LHS front and clear edge against back of slide holder
- 2. Using the 10x objective (and Kohler illumination) locate co-ordinates
- 3. Proceed to 40x or 50x objective and adjust focus as required (using oil if applicable). Locate co-ordinates and revert back to the 10x objective.
- 4. Taking care not to alter the position of the fixed stops of the stage, remove the graticule and replace with the slide of interest.
- 5. Proceed stepwise to 40x or 50x objective (oil or dry as applicable)
- 6. Adjust focus and locate sperm

NB: If the stage has moved repeat from step 1.





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# The Acid Phosphatase screening test for seminal stains

## 1 Purpose and scope

This method describes the screening test for the possible presence of human seminal fluid. This screening test is a presumptive test that detects acid phosphatase (AP) activity as an indicator of human seminal fluid. The microscopic identification of spermatozoa confirms the presence of human seminal fluid.

### 2 Introduction

Whole Semen is a suspension of cells and spermatozoa in a fluid medium called the seminal plasma. The average volume of human ejaculate is approximately 3mL, with a sperm density of 100 000 cells/µL which represents about 10% of the total volume.

The seminal plasma is rich in non-enzymic and enzymic constituents. In particular, it has an extraordinarily high phosphatase activity; i.e. phosphatases (acid and alkaline), nucleotidases, pyrophosphatases and several ATP'ases, and is the richest known source of acid phosphatase. Freshly ejaculated semen is also rich in phosphorylcholine, which is immediately dephosphorylated by acid phosphatase to choline and orthophosphate.

In this laboratory detection of possible human semen and seminal stains is demonstrated by the presence of acid phosphatase.

The test for acid phosphatase described below is a versatile, simple and relatively cheap method used only as a screening test for human seminal stains. The presence of acid phosphatase is not specific for human semen. Acid phosphatase activity is found in moderate amounts in some vegetable extracts, yeasts, fungi, and bacteria. Acid phosphatase activity may also be found in faecal matter, though this is usually distinguishable from seminal acid phosphatase activity by the suspect colour of the azo-dye formed. In addition, free phenols in a stain e.g. from contraceptive creams may also react with the Fast Blue though again usually to give an azo-dye of suspect colour. Animal semen contains acid phosphatase though generally at low levels. Most importantly, vaginal fluid also contains moderate levels of an acid phosphatase which displays similar chemical properties to seminal acid phosphatase. The level of acid phosphatase on vaginal swabs can be sufficiently high to cause confusion between pure vaginal secretion and weak seminal contamination. The level of acid phosphatase in vaginal secretions is thought to rise during pregnancy and may be affected by the menstrual cycle.

Conversely, the absence of acid phosphatase activity from a stain does not necessarily mean that the stain is not of seminal origin. Seminal acid phosphatase originates in the prostate gland. The other glands of the male genital tract secrete little or no acid phosphatase. Thus obviously, males who have undergone prostatectomy may have a decreased amount of acid phosphatase in their semen. Since different parts of an ejaculate originate in different glands, it is also possible for one seminal stain, say on a sheet, to differ markedly in its acid phosphatase content from a second stain from the same ejaculate. It has also been shown that acid phosphatase activity can be lost from a garment after most washing procedures whereas spermatozoa can persist through all but the most rigorous of

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such procedures. Finally, acid phosphatase activity in the vagina is lost faster than spermatozoa.

Within these limitations, the acid phosphatase test is an excellent screening test for seminal stains, and is far superior to visual examination which will miss all but the heaviest stains. However the acid phosphatase test can exhaust biological material. This means DNA, if present, may be lost through the actions of the test itself. It is advisable to perform tapelifts on areas of interest (e.g. crotch of underwear), prior to acid phosphatase testing.

## 3 Principle

Acid phosphatase activity in a stain is a strong indication of the presence of seminal fluid. It is a water soluble enzyme and is therefore easily absorbed onto moistened filter paper where it can be detected using the Brentamine test.

### 3.1 The Brentamine Reaction

This reaction relies on the liberation of napthol from sodium  $\alpha$ -naphthyl phosphate by the enzyme, and the concomitant formation of a purple azo-dye by the coupling of napthol with buffered Brentamine fast blue B.

## 4 Reagents and equipment

## 4.1 Safety

WARNING: The AP solution is carcinogenic – take great care when handling the reagents and wear gloves the whole time. Do not inhale mists.

NOTE: Any reagents prepared in-house shall bear a label created at the time of preparation:

```
....(enter details eg 10% NaOH)....
Prepd from Lot/batch:.....

Date: ../../. Initials: .....

Expires:../../. Store at:....°C

WARNING: Contains ......
```

## Brentamine Fast blue B (Sigma D-3502).

WARNING: Fast Blue Salt BN (o-dianisidine, tetrazolized) is harmful by inhalation, contact with skin, or if swallowed. Possible carcinogen, possible mutagen. Do not breathe dust. Wear PPE.

## Sodium Acetate (Anhydrous, AR).

WARNING: Sodium acetate may irritate eyes and repeated exposure may cause dermatitis. Wear eye protection and gloves.

## Acetic Acid (glacial, AR).

WARNING: Glacial acetic acid. Vapours, liquid mists are extremely corrosive to eyes, skin, respiratory surfaces and membranes. Avoid contact, do not inhale or ingest. Wear PPE.

## Sodium α-naphthyl phosphate (Sigma N-7255).

WARNING:  $\alpha$ -naphthyl phosphate di-sodium salt is irritating to the eyes, respiratory surfaces and skin. Wear PPE.



Approver/s: Cathie ALLEN

## Nanopure water

Nil.

## 4.2 Preparation of Working Reagent

Prepare Na  $\alpha$ -naphthyl phosphate by dissolving 0.8g in 10mL nanopure water. This acts as a stock solution. Reagent should be protected from light, when mixing wrap schott bottle in foil.

To about 300mL of Nanopure water add:

- 1. 4.8g Anhydrous Sodium acetate and
- 2. 0.4g Brentamine Fast Blue B Salt (Swirl to dissolve)
- 3. 4mL of Na α-naphthyl phosphate
- 4. Add 10mL of Acetic Acid
- 5. Make up solution to 400mL with Nanopure water
- 6. Decant into 2ml tubes and label each tube individually as per section 4.1. The reagent can also be decanted into 50ml falcon tubes for larger items.
- 7. Store the tubes in the freezer in a light protective plastic box

NOTE: If the reagent is not frozen it can be stored in the fridge and reused for 7 days. Any reagent that is frozen must have a 6 month expiry date and be discarded after this time.

The reagent is tested with positive and negative controls at time of solution preparation and prior to each use (as outlined in section 8). If no purple colouration develops with the positive control, discard the reagent. If a second batch of reagent also proves ineffective, refer to section 4.2 of SOP 30800 and/or notify Senior Scientist of Evidence Recovery Team.

Reagents are to be registered in the FR as per 34103 Receipt, Storage and Preparation of Chemicals, Reagents and Kits in Forensic Register.

## 5 Procedure

## 5.1 Acid Phosphatase screening of items

This process applies to whole items such as bedding, clothing, sanitary items or tissues. Determine the area of interest to be AP screened depending on the case particulars. For items of clothing and some bedding it will be necessary to keep front and back surfaces separate by sliding suitable perspex or other separators into the garment. The thickness of an exhibit material will govern whether one side or both sides require testing. Lightly spray the item with Nanopure water - take care not to over-moisten the item as this dilutes the stain.



- 1. Place a suitably sized piece of blotting or filter paper over the item or area in question and mark its position and orientation on the item with chinagraph or felt pen.
- 2. Spray the paper with Nanopure water take care not to over-moisten the paper as this makes further examination difficult.
- 3. Press the paper onto the item firmly so that any seminal staining will come into contact with the dampened paper.
- 4. Hang the paper in the fume cupboard with the side that has been in contact with the item facing out.
- 5. Spray the side of the paper, which contacted the item with tested AP solution.
- 6. Allow the process to run for 2 minutes and complete interpretation as per section Interpretation of Results below. A positive reaction is obtained if a purple colouration appears within 2 minutes (the stain may develop gradually). If a positive result is obtained, proceed to next step. If a negative result is obtained, this will either prompt further AP testing of the exhibit or signify that semen was not detected on the exhibit.
- 7. The side of the blotting paper which was in contact with the item needs to be facing up and placed on a clean area of bench. Cut out the AP positive area.
- 8. The blotting paper sheet is then placed back on the item and repositioned using the chinagraph marks. The AP positive areas are then traced back on to the item.
- 9. Place the used paper in the biological waste bin.
- 10. Further testing of any AP positive areas should be conducted at the discretion of the examining scientist as per SOP 17189 "Examination For & Of Spermatozoa" and or 17185 "Detection Of Azoospermic Semen in Casework Samples".

## 5.2 Acid Phosphatase of substrates within a tube

This process details the examination for the presence of semen on substrates received within tubes or placed in a tube as part of sample preparation. This includes, but is not limited to, swabs, tapelifts, scrapings and other textiles.

(**Note:** if sample has already been prepared for other testing, start at step 2 or 3as appropriate)



- 1. If the item is a swab cut the swab head from the stick, and then cut into several pieces and place in 1.5mL tube. If the item is a tapelift or fabric cut in smaller portions if required.
- 2. Add approx 200µL Nanopure water to the swab head, tapelift or fabric pieces or as little water as is required to cover the substrate. It is important to minimise the amount of water added to the substrate as the addition of water dilutes the sample. If the sample has already been processed for different testing, some water may already be present and less water may be required.
- 3. Mix the substrate suspension using a vortex mixer.
- 4. Spin down the substrate suspension using centrifuge set at 14000rpm for 3 minutes.
- 5. Retrieve a tube of AP reagent from the freezer and allow to defrost
- 6. Apply 1 drop of the substrate supernatant and 1 drop AP reagent drop-wise directly onto filter paper and interpret reaction as per 6.2. If a positive result is obtained, proceed to step 7. If a negative result is obtained, sample should be submitted for routine processing.
- 7. Further testing of any AP positive areas should be conducted at the discretion of the examining scientist as per SOP 17189 "Examination For & Of Spermatozoa" and or 17185 "Detection Of Azospermic Semen in Casework Samples

## 6 Results and Interpretation

## 6.1 Recording of Results

Test results must be recorded in the "Presumptive" test in the Forensic Register under the exhibit barcode the test has been performed on using the radio buttons. Positive results are to be accompanied with the time taken for the colour reaction to develop. Negative results however, do not require the time of the reaction to be noted, as a negative result is defined as no visible colouration development at 2 minutes.

## 6.2 Interpretation of Results

A positive AP reaction is recorded where a purple colouration, similar to the positive control develops within 2 minutes. Any stain producing a positive reaction should be considered as a potential seminal stain. A pure seminal stain may give an intense colour development in as little as 5 seconds. A weak seminal stain may take 60 to 90 seconds. Some substances other than seminal fluid will give delayed reactions from as little as 30 to 60 seconds. Keep a record of the time when the positive result developed. A negative AP reaction is indicated by no colour reaction within 2 minutes. A negative acid phosphatase test result does not mean that seminal fluid was not present, but only not detected.

## 6.3 False positive reactions

The following substances have been noted to give a positive reaction to AP testing:

Faecal stains – results with fast blue B reagent can be distinguished from a true seminal result in that the reaction is slower and fainter, and pink/brown in colour.

Vaginal secretions – vaginal acid phosphatase gives a slow, faint pinkish result with fast blue B reagent which can be similar in intensity to weak seminal stains. The activity is enhanced during pregnancy and when there is evidence of vaginal infection. The most common false positive result will be from vaginal secretions staining underwear.

Plants – positive but slow reactions with a brownish appearance are obtained from vegetable stains, e.g. cauliflower, sprouts, etc. tea may give a positive Brentamine reaction – this is due to the presence of phenols which combine with the dye.



Fungi – fungal acid phosphatase may cause a positive reaction.

Bacteria – positive results may be obtained when there is bacterial contamination. The bacterial content of the vagina is high during pregnancy and with vaginal infections; bacteria may also be present on unwashed fabrics (clothing/bedding).

Contraceptive creams – a brownish purple colouration is obtained with certain creams. This is not due to acid phosphatase, but to resorcinol (m-dihydroxybenzene) and a positive reaction is obtained with solution A of the Brentamine test. Other chemicals which combine with the diazo compound include  $\alpha$ -naphthol, 8 hydroxyquinoline, phloroglucinol.

False positive stains will be negative for spermatozoa or PSA.

## 6.4 Interpretation or estimation of time since deposition of semen

The best answer is "since the garment was last thoroughly washed." Seminal stains have been detected both in this and other laboratories on old (decades) dry fabrics stored at room temperature. There are however, some exceptions. Experiments have shown that very heavy seminal stains deposited into the crotch of a pair of heavy denim jeans may be sufficiently shielded from washing and full elution in a fully loaded machine wash.

Smears prepared from fabrics where heavy seminal stains have been present and the fabric washed have resulted in small numbers of spermatozoa seen even though the washing has eluted the seminal fluid (AP negative screening). It appears the spermatozoa can be trapped by the fibres while the soluble components elute into the wash.

## 7 Validation

This method is used universally in forensic laboratories and there are numerous peer reviewed published articles available. Within Forensic DNA Analysis the method of freezing aliquots of AP reagent for use in screening for seminal fluid has been validated in project #136.

## 8 Quality assurance/acceptance criteria

Controls are used to test the quality and validity of the reagent prior to use.

A positive and negative control should be tested prior to testing of exhibits, each time a new batch of working AP solution is prepared and when positive controls are prepared. A positive control is a known sample of human semen and Nanopure water is used as a negative control.

The positive control is known human semen on a piece of filter paper.

The negative control is Nanopure water on a piece of the same filter paper used to create the AP positive control with the absence of semen.



### 8.1 Creation of AP controls

Human semen is provided by a donor and collected in a sterile green-capped "Falcon" tube. The tube is to be labelled with the donor number and date of collection. The semen is to be stored within a freezer until required to create AP positive controls.

- 1. Clean down a fume cupboard bench using bleach and 70% ethanol solution and place portions of blotting paper onto the fume hood bench surface.
- 2. Using sterile forceps lay individual pieces of filter paper onto the blotting paper draw a circle in the centre of each filter paper with a black marker pen. Spot 1 to 2 drops of semen onto each circle using a disposable plastic pipette and allow to dry. The concentration of semen used may be neat; 1/2 dilution; or 1/5 dilution. See Table 1.

**Table 1 Semen Dilutions** 

Semen dilution	Semen volume (µL)	Nanopure water (μL)
neat	25	0
1/2	25	25
1/5	10	40

Each drop from a pasture pipette is  $\sim 50~\mu L$ . To make 40 AP controls for a 1/2 dilution use  $1000\mu L$  semen and  $1000\mu L$  Nanopure water for a 1/2 dilution. To make 40 AP controls for 1/5 dilution use  $400\mu L$  semen and  $1600\mu L$  Nanopure water.

 Package the AP positive controls into CSPBs labelled with the semen donor ID, initials of scientist preparing the positive controls and date prepared. The AP positive controls are to be stored in the freezer within Rm 6124.

## 8.2 Testing and interpretation of control samples

- 1. Remove an AP positive control from the freezer and allow to come to room temperature.
- Add a drop of the AP reagent to the positive control and interpret reaction after 2 minutes. A purple colouration develops within 5 seconds.
- 3. Add a drop of the AP reagent to the AP negative control and interpret the reaction after 2 minutes. No colouration should develop.

Positive control: a purple colouration develops within 5 seconds Negative control: does not develop a purple colouration within 2 minute



### 9 References

- Biology Methods Manual, London, Metropolitan Police Forensic Science Laboratory, 1978, pp 3-17 to 3-19.
- 2. Davies, A. and Wilson, E., (1974), "The persistence of seminal constituents in the human vagina" Forensic Sci., 3, 45-55.
- 3. Gutman, A. and Gutman, E., (1941), "Quantitative relations of a prostatic component (acid phosphatase) of human seminal fluid", Endocrinology, 28, 115-118.
- 4. Kaye, S., (1949), "Acid phosphatase test for Identification of seminal stains", J. Lab.Clin.Med., 34, 728-732.
- 5. Kind, S., "The Acid Phosphatase Test", in "Methods of Forensic Science" A. Curry Ed., London, Interscience, 1964, Vol III, pp 267-288.
- 6. Lundquist, F., (1946), Nature, 158, 710.
- 7. Prabhakaran, K., Rajamannar, K., Damodaran, C. and Visnatha Ras, V., (1981), "A rapid test for seminal stain acid phosphatase", J.Pol>Sci.Admin., 9, 76-79.
- 8. Spector, J. and Von Gemmingen, D., (1971), "The effect of washing on the detection of blood and seminal stains", Can. J. Forens.Sci., 4, 4-10.
- 9. Willott, G., (1972), "L-tartrate inhibitable acid phosphatase in semen and vaginal secretions", J. Forens.Sci.Soc., 12, 363-366.

### 10 Associated documents

QIS: 17185 Detection of Azoospermic Semen in Casework Samples

QIS: 17189 Examination For & Of Spermatozoa

QIS: 22857 Anti Contamination Procedure

QIS: 23849 Common Forensic DNA Analysis Terms and Acronyms

QIS: 34103 Receipt, Storage and Preparation of Chemicals, Reagents and Kits in Forensic Register

QIS: 33798 Examination of Sexual Cases (Forensic Register)

QIS: 33800 Examination of Items (Forensic Register)

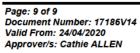
## 11 Amendment history

Revision	Date	Author/s	Amendments
0	Unknown	Unknown	
1	Unknown	Unknown	
2	03 Aug 2000	R Grice	
3	25 Nov 2002	V lentile	Update actions, method numbers
4	12 Jul 2006	J Howes/ A Williamson	Use of Polilight eliminated.
5	02 Aug 2007	N Govind	Approver to CJA.
QIS <sup>2</sup> Edition	n		
Version	Date	Updated By	Amendments
6	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland





-	10.14	140 "	
7	12 May	K.Scott	Remove reference to CrimeLite for
	2009		localization of stains. Altered the
			method of transferring AP positive
			areas from the blotting paper back
			on to the item. Change method for
			AP screening swabs to include the
			preparation of a suspension.
8	12 May	K.Scott	Version Correction
	2009		
9	30 June	K.Scott	Addition of associated documents
	2009		Add hyperlinks to QIS documents
			Remove PSA as a confirmatory test
			for seminal fluid – now a
			presumptive test.
			Clarify acceptance of test results
			and test interpretation.
			Add specific dates to amendment
			history
			Format changes
10	19 Jul 2012	M Brian	Changed Quality and project
			scientist to ERT scientist; section 9
			QC rewritten; added 30800 and
			23849 to associated documents.
			Changed section 7.1 reagent notes.
			Reworded sections of section 7 and
			9. Section 8 rewritten. Purpose and
			scope have been combined; fixed
			amendment history table; amended
			"introduction" to include reference to
			human semen and seminal fluid.
11	02/01/2014	M Brian	Updated template.
12	13/07/2015	A Houlding	New template. Formatting changes.
12	13/01/2013	Ariodiding	Corrected amendment history table.
			Added use of black marker pen
			when creating positive semen
			controls. Added SOP 32106 as an
		[	associated document. Changed
			preparation of working reagent
			method to incorporate freezing the
			reagent.
			Added validation (project #136).
42	20/00/0046	A Mahlaria	Other minor wording changes.
13	29/08/2018	A McNevin	Updated to reflect FR processing &
			workflow.
14	25/03/2020	A Morgan	Added light protection instructions
			for AP reagent. Updated Table 1
			semen dilution and added dilution
			amounts for 40 AP controls.





# Detection of azoospermic semen in casework samples

## 1 Purpose and scope

The purpose of this document is to describe the procedure for the presumptive detection of azoospermic semen. This method describes the ABAcard p30 test for Prostate Specific Antigen (PSA) or p30. To be used when a possible human seminal stain is suspected of having low or no spermatozoa (oligospermic / azoospermic). This procedure applies to all Scientists working within Forensic DNA Analysis performing testing. The principles of this procedure apply to all Scientists reporting on the findings of testing to clients, including the Courts.

## 2 Definitions

PSA: Prostate Specific Antigen

p30: Alternate nomenclature for PSAProcedure: specified way to perform an action

## 3 Principle

The ABAcard p30 kit has been designed for the detection of PSA/p30 in blood samples collected from patients with prostate cancer. Within this laboratory, the ABAcard p30 kit is used to detect the PSA / p30 component of seminal fluid. This test has been validated for use in this and many forensic laboratories. It uses a solid phase immunochromatographic format for the qualitative detection of p30. It has been validated for use with forensic casework stains and swabs.

The sample is added to a well in the test device containing a pad impregnated with a dye conjugated anti-p30 antibody. When sample is added to the well, it diffuses through a membrane where an anti-p30 antibody has been immobilised in a strip. If p30 is present in the sample at a concentration of  $4\mu g/L$  or more, a pink line will appear within 10 minutes in both the Test (T) and Control (C) areas where the coloured conjugate has been captured. If no band appears in the test window, the PSA concentrate is either less than  $4\mu g/L$  or not present in the sample. If no band appears in the control (C) window, the test is considered invalid and should be repeated.

Samples with very high levels of p30 may overload the test mechanism and prevent the antigen-antibody complex with the pink colour from binding to the antibody. As a result no pink line will form in the Test (T) area although p30 is present. This false negative result is known as the 'high dose hook effect'.

Samples that are strongly acid phosphatase positive (i.e. under 20 sec) with no spermatozoa detected and a negative p30 test should be suspected of the 'high dose hook effect'. To test whether the effect has occurred, the supernatant should be diluted and retested using the ABAcard p30 test

PSA /p30 is a major protein present in seminal fluid, it is produced in the epithelium of the prostate gland and is secreted into semen. However, other parts of the body such as the paraurethral glands, perianal glands, apocrine sweat glands and mammary glands also have traces of PSA. It is for this reason that small quantities of PSA/p30 can be detected in urine, faecal material, sweat and breast milk as well as low levels present in blood. In

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particular, elevated PSA/p30 levels are present in the serum of individuals with prostate cancer. [include reactions, where applicable]

## 4 Reagents and equipment

## 4.1 Reagents

ABAcard p30 test kit cassette Nanopure water

## 4.2 Equipment

Pipette
Vortex tube mixer
Centrifuge
1.5 mL sample tube or 2.0 mL sample tube
Timer

## 5 Safety

There are no special safety risks associated with this method

### 6 Procedure

## 6.1 Performing the test

- Remove test cassette from sealed foil pouch immediately prior to use and label accordingly.
- 2. Add 150 μL of the sample supernatant to the sample well "S". If capillary action doesn't occur in the test strip within 30 sec, add one drop of nanopure water.
- 3. Read test result in 10 minutes.
- 4. If the test result is difficult to read (i.e. test line is not distinct), have another scientist verify the test result.
- 5. Record p30 batch / lot number into LIMS see QIS: 33798 Examination of Sexual Cases for the entry of test result and appropriate result lines.

## 6.2 Diluting the supernatant for suspected "high dose hook effect" samples

- 1. Remove 20 μL of supernatant from the original suspension using a pipette and add to a new 1.5 mL tube.
- Add 180 μL of nanopure water to the tube. This creates a 1:10 dilution of the original sample.
- 3. Vortex mix thoroughly.
- Perform test as per Section 6.2.

**Note:** A 1:10 dilution should negate samples with possible 'high dose hook effect'. If unsure, a 1:100 dilution may be prepared and tested.



### 7 Validation

The ABAcard p30 test kit has been validated for use in 2011 within Forensic DNA Analysis (reference below).

## 8 Quality assurance/acceptance criteria

Refer to "Principle" above for test specific QA criteria. Each delivery (or each different lot number within a delivery) is QC tested using a known semen sample (positive control) and nanopure water (negative control) to test the integrity of the delivered kits.

## 9 References

ABAcard p30 Test for the Forensic Identification of Semen Technical Information Sheet (Abacus Diagnostics, Inc.) (Catalogue No: 308332).

Manfred N. Hochmeister, M.D et al. 'Evaluation of Prostate-Specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid' Journal of Forensic Sciences. 1999 Sep 1; Volume 44, Issue 5: pg 1057-1061.

Kaye, S., (1949), "Acid phosphatase test for Identification of seminal stains", J. Lab.Clin.Med., 34, 728-732.

Kind, S., "The Acid Phosphatase Test", in "Methods of Forensic Science" A. Curry Ed., London, Interscience, 1964, Vol III, pp 267-288.

Prabhakaran, K., Rajamannar, K., Damodaran, C. and Visnatha Ras, V., (1981), A rapid test for seminal stain acid phosphatase, J.Pol. Sci. Admin., 9, 76-79.

Biology Methods Manual, London, Metropolitan Police Forensic Science Laboratory, 1978, pp 3-17 to 3-19.

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Gutman, A. and Gutman, E., (1941), Quantitative relations of a prostatic component (acid phosphatase) of human seminal fluid, Endocrinology, 28, 115-118.

Spector, J. and Von Gemmingen, D., (1971), The effect of washing on the detection of blood and seminal stains, Can. J. Forens.Sci., 4, 4-10.

Davies, A. and Wilson, E., (1974), The persistence of seminal constituents in the human vagina. J Forensic Sci., 3, 45-55.

Parry, R., Lee, K., Keating, J. and O'Neil, K., (2009), Potential Sources of False Positive Results in the Biosign Prostate-Specific Antigen (PSA) Test Kit. QHFSS Internal Document.

Khaldi, N., Miras, A., Botti, K., Benali, L., Gromb, S. (2004) "Evaluation of Three Rapid Detection Methods for the Forensic Identification of Seminal Fluid in Rape Cases." NB: Controls are incorporated in the device. See 7.3. Journal of Forensic Sciences July; 49(4):749-753.

Maher, J., Vintiner, S., Elliot, D., Melia, L. (2002) "Evaluation of the BioSign PSA Membrane Test for the Identification of Semen Stains in Forensic Casework." The New Zealand Medical Journal Feb 8:115(1147):48-49.



Lloyd, A., Pippia, A., Nurthen, T., Scott, K. (2011) "Report – Verification of ABAcard p30 Test'. QHFSS Internal Document.

Li, Richard (2008) Forensic Biology, CRC press, 8.1.3 Prostate-Specific Antigen (PSA) pp117

## 10 Associated documents

QIS: 17186 - The Acid Phosphatase Screening Test for Seminal Stains

QIS: 17189 - Examination For & Of Spermatozoa

QIS: 22857 - Anti-Contamination Procedure

QIS: 23849 - Common Forensic DNA Analysis Terms and Acronyms

QIS: 30800 - Investigating Adverse Events in Forensic DNA Analysis

QIS: 33798 - Examination of Sexual Cases

QIS: 33800 - Examination of Items



## 11 Amendment history

Revision	Date	Author/s	Amendments					
0	27 Jul 2000	V lentile						
1	18 Nov 2003	L Freney	Republish					
2	14 Oct 2006	S Cave	Approver changed, password protection removed.					
3	25 Oct 2007	N Govind	More references added.					
0	27 Jul 2000	V lentile						
QIS <sup>2</sup> Edition								
Version	Date	Updated By	Amendments					
5	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland					
6	October 2009	V Caldwell	Update of methods / procedures.					
7	March 2010	V Caldwell	Addition of Reference and Associated Documents.					
8	July 2011	A Lloyd	Major revision: change use of Biosign PSA to ABAcard p30. Updated methods/principle/ interpretation, included dilutions for negating high dose hook effect. Updated references.					
9	September 2013	V Caldwell	Updated to HSSA headers and footers. Added "human" seminal stain to scope. (p30 is interchangeable with PSA) in reagents. Changed 2.4 reference to section 8. Removed 4 @ bottom of document					
10	April 2015	A McNevin	Updated to new HSQ / FSS template, minor wording adjustments and movement of text to suit new template					
11	May 2018	A McNevin	Update to reflect FR changes, added to principle, updated references					
12	July 2021	A McNevin	Updated to new template, removed reference to testing after AP, removed process for creating supernatant (now covered in QIS 17189)					

## 12 Appendices

Nil



# **Queensland Health**

# Forensic and Scientific Services

Sexual Assault Investigation Kit (SAIK) Forensic DNA Analysis, Forensic and Scientific Services, Department of Health								
SURNAME: GIVEN NAMES:								
DOB: SEX: Male   Female								
Examining Doctor / FMO / FNE: Investigating Officer:								
Address of examiner: Police Station:								
E-mail address: E-mail address:								
Contact Phone Number: Contact Phone Number:								
Date of SAIK Examination: Date of Assault:								
Time of SAIK Examination: Time of Assault:								
Sterilisation process complete for workspace & equipment Yes  No  Sterilisation process complete for workspace & equipment Yes No Sterilisation process complete for workspace & equipment Yes								
TYPE OF ASSAULT:    Digital   Penile penetration   Oral penetration   Oral penetration   PREVIOUS SEXUAL ACTIVITY: Yes   >7 days previous								
Oral								
Vaginal Was previous coitus with suspect? Yes ☐ No ☐ Unknown ☐								
Anal Was condom used with previous coitus? Yes No Unknown								
Penile For Female SAIK								
Other (please provide details in "Summary of Assault"):  Other (please provide details in "Summary of Assault"):  Date of last LMP:								
ASSAULT DETAILS CASE HISTORY								
Was a condom used: Yes No Unknown Has the patient consumed a substance which may alter consciousness?								
Did ejaculation occur: Yes No Unknown Yes No Unknown Unknown								
Was a lubricant used: Yes No Unknown Can the patient recall and communicate the incident?								
(if yes, please provide details in summary of assault details section)  Yes No Unknown								
Did the assault involve any kissing / licking / biting?								
How many offenders were involved: Male Female Yes No Unknown (If multiple offenders or if unknown, please detail within summary of If yes, locations:								
assault details section)  Was there any genital / anal injury causing bleeding?								
Yes No No								
SINCE THE ASSAULT, HAS THE PATIENT:  If yes, details:								
Changed Clothing: Yes No Douched: Yes No								
Washed Clothing: Yes ☐ No ☐ Urinated: Yes ☐ No ☐								
Showered / Bathed: Yes No Defecated: Yes No No Defecated: Yes No No Defecated: Yes N								
Washed Mouth: Yes ☐ No ☐ Vomited: Yes ☐ No ☐								
Cleaned Teeth: Yes No Had drink / food: Yes No No								
SUMMARY OF ASSAULT DETAILS (please provide as much information as possible as this assists with DNA Analysis):								
(continue on next page)								

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Sexual Assault Investigation Kit (SAIK) Forensic DNA Analysis, Forensic and Scientific Services, Department of Health							
SUMMARY OF ASSAULT DETAILS (	continued from previous):						
-							
-							
-							
CLOTHING AND OTHER ITEMS							
Item Clothing / Sanitary Items: Details:							
Worn pre-assault: □	Worn post-assault □	Worn pre- and post-assault □					
Item Clothing / Sanitary Items:							
Details:							
Worn pre-assault: □	Worn post-assault □	Worn pre- and post-assault □					
Blood sample taken for Toxicology?	Yes  No	Urine sample taken for Toxicology?	Yes 🗌	No 🗆			

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Sexual Assault Investigation Kit (SAIK) Forensic DNA Analysis, Forensic and Scientific Services, Department of Health							
Please o	Please document the samples collected for DNA below						
	SAIK SWABS:						
	High vaginal			Low vaginal			
	Vulval			Cervical			
	Perianal			Rectal			
	Oral			Other			
	Other			Other			
MALE S	AIK SWABS:						
	Base of penis			Shaft of penis			
	Glans penis			Scrotum			
	Perianal			Rectal			
	Oral			Other			
	Other			Other			
BODY/S	KIN SWABS: (please specify if biting, licking	or kissing has occurr	ed at site f	from which body	y swab was collected)		
	Location			Location			
	Location			Location			
	Location			Location			
	Location			Location			
	Location			Location			
FINGER	NAIL CLIPPINGS/SCRAPINGS:				DROP SHEET		
	Left Hand	Right Hand					

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## Forensic and Scientific Services



# Configuration of SAIKS (Sexual Assault Investigation Kits)

#### 1 Purpose

To describe how the Sexual Assault Investigation Kits (SAIKs) are prepared within Forensic DNA Analysis.

#### 2 Scope

This procedure describes the preparation of the SAIK within Forensic DNA Analysis for all Forensic DNA Analysis staff.

#### 3 Explanation

SAIK's are prepared on site in Room 6117 within Forensic and Scientific Services, Forensic DNA Analysis. Completed SAIK's are stored in Forensic DNA Analysis SAIK room (Rm 6110)

Two types of SAIKs are compiled by Forensic DNA Analysis:

- 1. **Generic SAIKs** these SAIKs are distributed to the *Child & Sexual Assault Investigation Unit (CSAIU)* of the Queensland Police Service and to the Forensic Medical Officers. These units distribute the kits to police as required.
- Just in Case (JIC) SAIKs these SAIKs are distributed to Pathology Queensland Laboratories and are to be used in instances where a patient has disclosed an alleged sexual assault but are not ready to involve police. A forensic examination can be requested "Just in Case" a police complaint may be made at a later date.

#### 4 Actions

#### 4.1 Generic SAIKs

These shall consist of a **clear** Tamper Evident Security bag with the following contents:

1.	One DNA Analysis address label
2.	One "Sexual Assault Investigation kit" label
3.	One large clipseal bag (30 x 23cm) containing
	- One "Medical Examination Information form" (QIS 31281)
	- One "Sexual Assault Toxicology form" (QIS <u>29066</u> )
4.	6 x Plain labelled swabs
5.	One large clipseal bag (30 x 23cm) labelled "Dropsheet" containing:
	One dropsheet (A1 sheet of paper folded to A4 size)
	"Directions for collection of Samples", dropsheet form

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#### Refer to Appendix D for direction on the preparation of the generic SAIK

#### 4.2 Creation of Labels for Generic SAIKs

Labels can be printed from the Zebra designer software on a GX430t label printer. The required labels have been designed as per follows:



Figure { SEQ Figure \\* ARABIC } - SAIK "Sexual Assault Investigation Kit" Label



Figure 2 - SAIK Dropsheet label



Figure 3 - Forensic DNA Analysis Address label

#### 4.3 Just in Case (JIC) SAIKs

These shall consist of an **opaque/white** Tamper Evident Security bag with the following contents:

One orange "CSR STAFF: DO NOT OPEN" label (attached to bag)					
One plastic document wallet sticker containing					
- One Pathology Queensland JIC Request Form					
- One Pathology Queensland Chain of Custody Form					
- One green Scientific Services address (adhesive intact, not attached*)					
One green Scientific Services address label (attached to bag)					
One pink Forensic Services label (attached to bag)					
One large clipseal bag (30 x 23cm) containing					
- One "Medical Examination Information form" (QIS 31281)					
- One "Sexual Assault Toxicology form" (QIS <u>29066</u> )					
6 x Plain labelled swabs					
One "Consent for Forensic Examination" Form					
One large clipseal bag (30 x 23cm) labelled "Dropsheet" containing:					
One dropsheet (A1 sheet of paper folded to A4 size)					
"Directions for collection of Samples", dropsheet form					

<sup>\*</sup>This is a spare label to be included – for later use on an esky

#### Refer to Appendix E for direction on the preparation of the JIC SAIK

Note – the unique barcode on the Tamper Evident Security bag is to be written on the Queensland Pathology Chain of Custody Form (in the "Satchel Identifier" field).

#### 4.4 Labels for JIC SAIKS

Pre-printed coloured labels (green and pink) will be received by Forensic DNA Analysis for the use on JIC SAIKs.

#### Orange CSR labels

Can be printed from the Zebra design software on a GX430t label printer by using the green cog wheel in the printer to open the label holder to max then feeding the orange labels through the back of the machine (manual hold and feed).

Go to a PC and from the Control Panel select "Devices and Printer Settings", right click on a ZDesigner printer and select "Printing Preferences" > "Advanced Set Up"> "Calibrate". The labels can then be printed from the Zebra design program.



Drop sheet labels can be printed from the Zebra designer software on a GX430t label printer, with the design as following:



Figure 4 - SAIK Dropsheet label

#### 5 Records

Nil

#### **Associated Documentation**

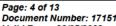
- QIS: 29066 Sexual Assault Toxicology Form
- Medical Examination Information Form DNA QIS: 31281
- Pathology Queensland JIC Request Form (Refer Appendix A)
- Pathology Queensland Chain of Custody Form (Refer Appendix A)
- Pathology Queensland Consent for Forensic Examination Form (Refer Appendix A)

#### 7 References

Nil

#### **Amendment History**

Version	Date	Author/s	Amendments
1	Unknown	Unknown	First Issue
2	26 Jun 2001	V lentile	Unknown
3	9 Jan 2004	V lentile	Change document numbers to QIS numbers, remove mention of unique numbers on front of SAIKs
4	26 Jun 2006	M Gardam / A Storer	Updated to include Central Property Point & storage area for kits
5	30 Apr 2007	G Tucker	Updated as part of project activity. Addition of labels and documents as appendices.
5	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland





5	31 Mar 09	QIS2 Migration Project	Updated hyperlinks for QIS2 and changed revision to version
6	12 April 2011	A PIPPIA	Update = Purpose; removed all reference to slides and slide holders; addition of form 29066 to kit; updated associated documents; update to consumable; changed reference of Forensic Biology to DNA Analysis Unit; update to GoPrint information; fixed amended history.
7	03 Oct 2013	Michelle Margetts	Complete re-write of procedure.
8	13 April 2015	Michelle Margetts	Updated procedure and template
9	25 Oct 2016	Michelle Margetts	Minor changes to room storage location General update
10	17 July 2017	Michelle Margetts	Updated stickers for SAIK
11	25 Jan 2019	Kirsten Scott	Update hyperlinks, label printing, suppliers and minor text edits
12	23 July 2019	Kerry-Anne Lancaster	Addition of instructions for Just in Case (JIC) SAIKs
13	08 Oct 2020	Phillip McIndoe	Update details for JIC Kits
14	20 April 2022	Abbie Ryan	Update template. Minor wording edits.



#### 9 Appendices

- Appendix A List of Consumable Items required to prepare SAIKs
- Appendix B Example of Requests for SAIKs Register
- Appendix C Directions for Collection of Samples using Drop Sheet
- Appendix D Directions for the Preparation of the Generic SAIK
- Appendix E Directions for the Preparation of JIC SAIK

Appendix A - List of consumable items required to prepare the SAIKs

Item	Unit of measure	Supplier	Catalogue/SAP Number/Comments
A4 Tamper Evident Security Bag (Clear)	Box / 500	Tamper Evident	BAG_TEB340500
A4 Tamper Evident Security Bag (Opaque)	Box / 500	Tamper Evident	BAG_TEB340W500
Plastic Document Wallet (Packaging Envelope Adhesive Plain)	Box / 500	Winc	88632985
Plastic Bags, Transparent with Klick-Seal 230mmW x 305mmD	Pk / 100	FAMMIS item	129687
Swab Sterile Plain, MW104	Box / 100	FAMMIS item	10121650
Dropsheet, Paper White 1020 x 760mm A1-80gsm bond paper	As available	TJ's imaging centre	By quotation
Officemax Rubber Band No.33 500gram Natural Rubber Bag	500g	OfficeMax	1044877
Sexual Assault Toxicology Form (QIS – 29066)	Ea	Document located in QIS	Document located in QIS
Medical Examination Information Form (QIS - 31281)	Ea	Document located in QIS	Document located in QIS
Dropsheet Information Sheet (Appendix C)	As required	Document located in QIS	Photocopy
Pathology Queensland – JIC Request Form	Ea	Pathology Queensland	Download from website**
Pathology Queensland – Chain of Custody Form	Ea	Pathology Queensland	Download from website**
Pathology Queensland – Consent for Forensic Examination Form	Ea	Pathology Queensland	Download from website**

<sup>\*\*</sup>Website - https://qheps.health.qld.gov.au/hsq/forensics/response-to-sexual-assault

(Note – when printing from the Pathology Queensland Website – there may be slight alignment errors on the request form. All current printable copies of the forms have been saved to G:\ForBiol\Evidence Recovery & Quality\JIC SAIK\Current JIC Forms July 2019, and can be accessed and printed from here – ensure to check them against the version on the website prior to printing so the most up to date form is included in the kit).



## Forensic and Scientific Services

## Appendix B – Example of Requests for SAIKs Register

Date	No. of Kits	Released to:	Released to: Signature	Released by: Name	Released by: Signature
		Name:			
		Business Unit:			
		Name:			
		Business Unit:			
		Name:			
		Business Unit:			
		Name:			
		Business Unit:			
		Name:			
		Business Unit:			
		Name:			
		Business Unit:			

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## Forensic and Scientific Services

#### Appendix C - Drop sheet cover sheet

# DIRECTIONS FOR COLLECTION OF SAMPLES PLEASE READ CAREFULLY BEFORE TAKING SAMPLES

#### **DROP SHEET (A1 sheet paper)**

The drop sheet is to be used for the purpose of collecting samples in the following way:

The sheet is spread out and placed on the floor. The person being medically examined stands on the sheet while undressing. Material dislodged from the person's clothes and body hairs i.e. hairs, fibres, plant material and foreign matter will drop on to the sheet.

The sheet is spread out on the examination couch beneath the buttocks of the patient. Material dislodged from the pubic and surrounding regions will drop onto the sheet.

On completion of the examination, the sheet should be carefully folded and placed in a plastic bag provided.

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## Forensic and Scientific Services

Appendix D - Compilation of the Generic SAIK



#### STEP 1

You will require the following:

- Forensic DNA Analysis address label
- "Sexual Assault Investigation kit" label
- 1 x Tamper Evident Security Bag



#### STEP 2

Attach both labels to the Evident Security Bag

- Forensic DNA Analysis address label (adhere over barcode)
- "Sexual Assault Investigation kit" label



#### STEP 3

Tear off the top perforation from the top of the Evident bag.

\*the perforated piece can be thrown out

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Contents to be prepared to be put in SAIK

- Form: "Medical Examination Information form QIS 31281" form and "Sexual Assault Toxicology form QIS 29066". To be packaged within a large CSP
- 6 x Plain labelled swabs
- Form: "Directions for collection of Samples" Dropsheet form and 1 x dropsheet. To be packaged within a large labelled CSPB



STEP 5



#### STEP 5

Place all contents within "Evident Security Bag"

SAIK COMPLETE

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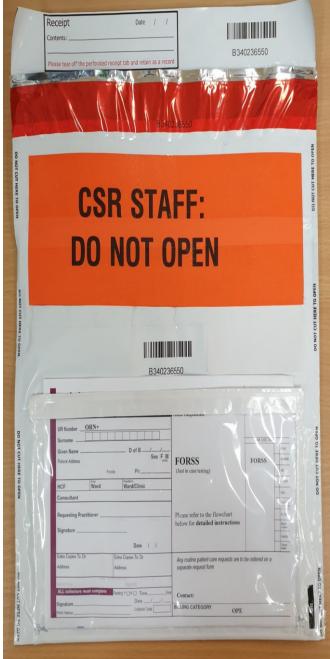
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## Forensic and Scientific Services

#### Appendix E - Compilation of the JIC SAIK

Front of Tamper Evident Bag



Orange label to be affixed to top of Tamper Evident Bag (above the unique barcode)

Plastic Document Wallet to be affixed towards the bottom of the Tamper Evident Bag (below the unique barcode)

One copy of the following to be placed inside the plastic wallet:

- Queensland Pathology Request Form
- Queensland Pathology Chain of Custody Form
- 1 Green address label (unused)

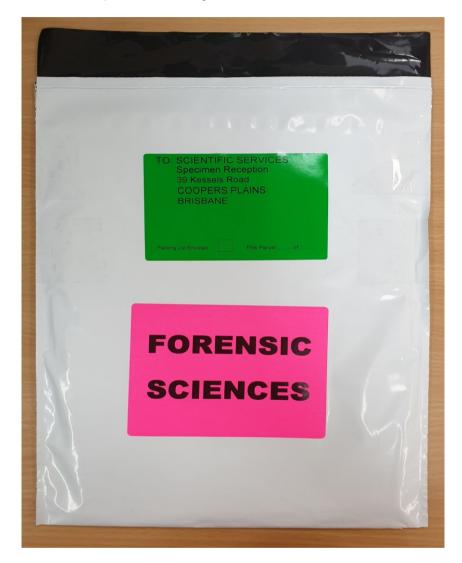
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## Forensic and Scientific Services

#### Back of Tamper Evident Bag



Green Scientific Services pre-printed label to be affixed towards the top of the Tamper Evident Bag

Pink Forensic Sciences pre-printed label to be affixed towards the bottom of the Tamper Evident Bag.

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## Forensic and Scientific Services



Once the labels and plastic document wallet have been affixed to the opaque/white Tamper Evident Bag, the following are to be placed inside the SAIK:

- 6 x Plain Labelled Swabs
- Large clipseal bag containing
  - o 1 x Medical Examination Information Form QIS 31281
  - o 1 x Sexual Assault Toxicology Form QIS 29066
- Large clipseal bag containing
  - 1 x Dropsheet
  - 1 x Directions for collection Samples Dropsheet Form (Appendix C)
- 1 x Forensic Examination Consent Form

The opaque/white Tamper Evident Bag can then be folded and placed inside a large (30 x 23cms) clipseal bag

The JIC SAIK is now complete

#### Spreadsheet for management of JIC SAIK Kit restocking:

https://teams.microsoft.com/l/channel/19%3a33f3693d1be740ac8b9c91d0e55c6c76%40thread.skype/General?groupId=0fccdb25-fe83-40df-86b4-5b1bc2abe716&tenantId=0b65b008-95d7-4abc-bafc-3ffc20c039c0

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# Health Support

Queensland

Forensic and Scientific Services

## Forensic DNA Analysis - Management Team Meeting

Date: 12 May 2016 Time: 9.30am – 11.45am

Venue: FSS CR611

1. Present

Allan McNevin (ARM) Amanda Reeves (AJR) Cathie Allen (CJA)

Kirsten Scott (KDS)
Justin Howes (JAH)

Kylie Rika (KDR)

Kerry-Anne Lancaster (KAL)

Luke Ryan (LBR)

Sharon Johnstone (SMJ) Wendy Harmer (WAH)

- 2. Apologies -
- 3. Guests Nil

#### Agenda items

Item	Торіс	
1.0	Confirmation of previous minutes - SMJ	
2.0	Conflicts of Interest - Nil	
3.0	Action Register – See below	
4.0	4.1 Workplace Health & Safety Issues — Annual safety audit of lab space to be done — allocated to a staff member on campus to complete. Vicky has completed a risk assessment of the freezer area — will be loaded to QIS soon. Details within the risk assessment.  4.2 Analytical Issues of Note - LBR 7500B has dye calibrations completed, 7500A to be done this morning. Hamilton instruments have been purchased and soon to be delivered to campus. No update on mastermix separation issue — other than LBR's email sent during this week. No comment back yet from PP21 BSAG labs.  4.3 AUSLAB Working Party Update — KDS A request for change regarding QIASymphony will need to be submitted (Quant Report Analyser Mask work).	

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#### 4.4 Project Updates

Project #152 - Y-Filer Plus - LBR.

No update from last meeting.

Project #168 - Validation of QIAsymphony - LBR

Running some final AS integrated runs (ie overnight & different temps). Report anticipated to be completed in the next week. Training in the next few weeks.

Project #170 – Reassessment of in-house stutter thresholds and stutter file used in STRmix – JAH.

Linked with v2.4 of STRmix. AAP and EJC looking for samples that can be re-used for v2.4. Need to coordinate any changes with implementation with v2.4 being brought online.

Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega) – I BR

Presentation and discussion ensued. LBR to send out an email with clarifying email and a voting question to respond to.

Project #172 – Phadebas testing from suspension in ERT – ARM

Report has been provided to Management Team for review. ARM would like to investigate the way that suspensions are made, so this project will be placed on hold until this has been completed. ACTION: ARM to look at the way in which suspensions are made.

#### Projects on-hold

Project #146 - GlobalFiler - LBR

On hold until new size standard has been completed (17.03.2016)

#### 5.0 New business

#### 5.1 LOD and LOR 3130x/ PP21 WEN (LBR)

As above. Presentation saved here: G:\ForBiol\DNA Analysis Team Meetings\DNA Analysis Management Team\2016\Jan - Jun

#### 5.2 HR – part day absences (time claimed) (CJA)

Please ask team members to ensure their leave taken is to the 15min, 30mins, 45mins or the hour as this requires resources to check on.

#### 5.3 OQI 41850 re: Link not being associated and reported (SMJ)

Link #12541 was found in 2012, however not placed into Auslab and reported to QPS. QPS have been notified. This was found due to the daily audit which was put in place after the last incident was found. Link was crime scene to crime scene, and when updated recently it was to add a Person Sample and audit discovered the missing crime scene profile – refer to OQI for detail.

#### 5.4 Sperm seen on Diff Lysis extraction slide vs ER suspension slide - ARM

This has been raised as a potential issue. First step to look at this is to compare a number of Diff Lysis extraction slides against ER suspension slide to see if the trend shows that more are seen on the extraction slides as a norm. This first step project is being monitored by ARM and KDS.



6.0 New business – for noting

Nil

**Next Meeting** 

Date: 26 May 2016, 9.30am

Venue: CR611

#### **ACTION REGISTER**

Minutes Reference	Item Number	Subject	Action	Action Officer	Status
14/04/2016	5.2	Validation Baseline Methods	To be prepared - one page on software, benefits, costs, if more than 1 product etc. Once software has been looked at, then this will affect the SOP to be created – Found STR-validator program (G:\ForBiol\DNA Analysis Team Meetings\DNA Analysis Management Team\2016\Jan – Jun) – SMJ to complete a project initiation document	SMJ	Closed
29/04/2016	4.1	Change in access for retrieving liquid nitrogen	Add a comment to the SOP	KDS	Closed





# Health Support

Queensland Forensic and Scientific Services

## Forensic DNA Analysis - Management Team Meeting

Date: 27 May 2016 Time: 9.30am – 11.00am

Venue: FSS CR611

1. Present

Allan McNevin (ARM) Kerry-Anne Lancaster (KAL)

Cathie Allen (CJA) Luke Ryan (LBR)

Kirsten Scott (KDS) Sharon Johnstone (SMJ)
Kylie Rika (KDR) Wendy Harmer (WAH)

2. Apologies - Justin Howes (JAH), Amanda Reeves (AJR)

3. Guests - Deb Whelan (DAW)

#### Agenda items

Agenda		
Item	Торіс	
1.0	Confirmation of previous minutes - SMJ	
2.0	Conflicts of Interest - Nil	
3.0	Action Register – See below	
4.0	Standing items  4.1 Workplace Health & Safety Issues — OH&S will be assessing a workstation to attempt to identify any potential risks. Strut has been replaced on the centrifuge which had previously caused a minor injury.  4.2 Analytical Issues of Note - LBR Nil — other than MPIIs which are still causing issues.  4.3 AUSLAB Working Party Update — KDS Have had a few service call submissions — ie prep for QIASymphony  4.4 Project Updates  Project #152 — Y-Filer Plus — LBR. ALL has prepared the written part for the mixtures and forwarded to AJR & KDR for peer review.	
	Project #168 – Validation of QIAsymphony – LBR  Training on the instrument has commenced and final touches to the report.	

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Project #170 – Reassessment of in-house stutter thresholds and stutter file used in STRmix – JAH.

Report has been sent for peer review by management team – to be completed by  $3^{rd}$  of June.

Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega) – LBR

3500 part has not been progressed due to other parts of the project. Mixtures have been assessed by EJC, however still uncertain about the baseline due to the previous thresholds. Have completed the Saturation plate and have run the 10 samples used in baseline plates (have reamped at 0.6 and 0.7ng) to be included in baseline data set – to see the effect on the calculated baseline – which is currently being read by LBR. Anticipate getting this out to management team by today or Monday 30<sup>th</sup> May. The additional amps have been covered by a one page amendment document by EJC. Have 55 tubes of CC5 – which will last about 4 weeks, so this is a time critical project that needs to be progressed as a priority.

Project #TBC Hamilton Instruments being validated - LBR

2 instruments delivered today (1 to come) – pre-PCR and CE instruments – training begins in about 2 weeks. Validation project will commence after training. The 3<sup>rd</sup> instrument to be delivered in June – and will be used for Pre-PCR.

Sperm seen on Diff Lysis extraction slide vs ER suspension slide - ARM

Discussed graphs and results produced so far, all agreed that ARM would initiate a project plan for the next step.

#### Projects on-hold

Project #146 - GlobalFiler - LBR

On hold until new size standard has been completed (17.03.2016)

Project #172 - Phadebas testing from suspension in ERT - ARM

Pending outcome of project on how suspensions are made. (12/05/2016)

#### 5.0 New business

**5.1 Risk Assessments - KDS** As a result of Audit#21986 (late December 2015) - in the January Management Team meetings we allocated risk assessments to each of the teams for completion.

According to QIS there are still are number outstanding. (Open Link Doc.)
ForBiol\DNA Analysis Team Meetings\DNA Analysis Management Team\2016\Jan Jun\Risk Assessments\_2016.xls

ACTION: JAH to advise KDS the staff member assigned to each of the FRIT allocated risks.

#### 5.2 FR Go Live for Forensic Chemistry – CJA

Brief overview provided of how it went with the team.

#### 5.3 Maternity Leave Backfill of HP4 – KDR

Cassandra James will be joining FRIT on the 11<sup>th</sup> of July due to Maternity Leave of 3 staff members.

#### 6.0 New business – for noting

## **Next Meeting**

Date: 9 June 2016, 9.30am

Venue: CR611

#### **ACTION REGISTER**

Minutes Reference	Item Number	Subject	Action	Action Officer	Status
14/04/2016	5.2	Validation Baseline Methods	SMJ to complete a project initiation document	SMJ	Closed
12/05/2016	4.4	Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega)	An email (including clarification) is to be sent out with voting options	LBR	Closed
12/05/2016	4.4	Project #172 – Phadebas testing from suspension in ERT	ARM to look at the way in which suspensions are made.	ARM	Closed
12/05/2016	5.4	Sperm seen on Diff Lysis extraction slide vs ER suspension slide	Project will be monitored by ARM and KDS.	ARM / KDS	Closed





# Health Support

Queensland Forensic and Scientific Services

# Forensic DNA Analysis - Management Team Meeting

9 June 2016 Date:

Time: 9.30am - 11.00am

Venue: FSS CR611

1. Present

Allan McNevin (ARM) Amanda Reeves (AJR) Deborah Whelan (DAW) Justin Howes (JAH) (Chairperson) Kirsten Scott (KDS)

Kylie Rika (KDR) Kerry-Anne Lancaster (KAL) Sharon Johnstone (SMJ) Wendy Harmer (WAH)

- 2. Apologies - Cathie Allen (CJA), Luke Ryan (LBR)
- 3. Guests - Pierre Acedo (PA), Allison Lloyd (AKL)

#### Agenda items

Item	Торіс	
1.0	Confirmation of previous minutes – SMJ	
2.0	<b>Conflicts of Interest</b> – Nil. Agenda sent out prior to meeting, if any conflicts exist, these are to be discussed with chair prior to meeting.	
3.0	Action Register – See below	
4.0	<ul> <li>Standing items</li> <li>4.1 Workplace Health &amp; Safety Issues – <ul> <li>Medical incident Tues – Incident form submitted to Deb.</li> <li>Bone room – door opens inwards. Given risks of staff passing out and blocking door, work to look into for door to open outwards.</li> </ul> </li> <li>4.2 Analytical Issues of Note - LBR <ul> <li>Nil of note.</li> </ul> </li> <li>4.3 AUSLAB Working Party Update – KDS</li> <li>Nothing – to be removed from next agenda.</li> </ul> <li>4.4 Project Updates <ul> <li>Project #152 – Y-Filer Plus – LBR.</li> </ul> </li> <li>Mixture elements in progress in FRIT.</li>	

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Project #168 – Validation of QIAsymphony – LBR

Maria training PA and BM. Report about to be completed. TMs and SOPs in QIS.

Project #170 – Reassessment of in-house stutter thresholds and stutter file used in STRmix – JAH.

Plan in review and related to STRmix v2.4.

Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega) – I BR

Extraordinary meeting established thresholds and 3500xL. Extracted refs being determined.

Project #173 & #175 Hamilton Instruments being validated – LBR

#173 – Installation has occurred. Training in coming week from manufacturer.

#175 - CE setup. Plan not finalised.

Project #181 - Sensitivity of Sperm microscopy - ARM

Plan in draft, background being worked on. Preliminary discussions on potential content discussed with no decisions made.

#### Projects on-hold

Project #146 - GlobalFiler - LBR

On hold until new size standard has been completed (17.03.2016)

Project #172 - Phadebas testing from suspension in ERT - ARM

Pending outcome of project on how suspensions are made. (12/05/2016)

#### 5.0 New business

#### 5.1 Sub-team whip around

- -OO: destructions lists in AUSLAB to be completed leading up to FR. Key task before FR.
- Intel: Audit NCIDD and AUSLAB approx. halfway through, 13 groups with information where not reported, nothing new in last two weeks, now looking at DNA numbers involved. Started with 23000 groups 13 of half this is not high %.
- Analytical: Many projects underway, temps extended.
- Admin: nothing significant to share, preparing HR for next year. NB. Thanks given to WAH for HP4 recruitment assistance.
- ER: effort at the moment into FR SOPs.
- R1/2: Mat leave and absenteeism issues at the moment pressure on allocations. Sharing staff between R1/2 required. Looking forward for new staff in next few months.
- **5.2 Ethical considerations** ARM met with Charles could bladder swabs and toenails assist with profiles from deceased, rather than bones? Charles asked if we could request this. Deb will follow up on what might be required here.

#### 5.3 Stress

Seem to have some stress around the lab at the moment, so please watch out for each other. Help provided to JAH of late has been appreciated.

#### 6.0 New business – for noting

Nil



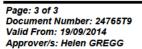
## **Next Meeting**

Date: 23 June 2016, 9.30am

Venue: CR611

#### **ACTION REGISTER**

Minutes Reference	Item Number	Subject	Action	Action Officer	Status
27/05/2016	4.4	Y-Filer Plus	Peer Review of Anna's written part	AJR / KDR	Ongoing – to be allocated to AAP.
					NB. Post meeting, JAH informed Anna's information has been reviewed and ready for inclusion.
27/05/2016	4.4	In house stutter thresholds	Report has been sent for peer review by management team – to be completed by 3 <sup>rd</sup> of June.	All of Mgmt Team	Ongoing
27/05/2016	4.4	Diff Lysis Extraction Slide vs ER Suspension slide	Initiate Project Plan	ARM	#181 created and Plan in draft
27/05/2016	5.1	Risk Assessments	Advise KDS of the name of FRIT staff members assigned to these assessments	JAH	2 x staff member conducted training with ERQ member mentoring. 1 x RA to be allocated.







# HealthSupport Queensland Forensic and Scientific Services

## **Examination of items**

#### 1 Purpose

The purpose of this procedure is to describe the procedures for the examination of evidentiary items by Evidence Recovery scientists and technicians in Forensic DNA Analysis.

#### 2 Scope

This procedure applies to all Forensic DNA Analysis staff that examine or interpret examinations of evidentiary items. This standard operating procedure is an adjunct to individual methods for particular screening tests. Interpretations and limitations of reporting are to be found in each method.

#### 3 Definitions

Refer to QIS document 23849 (Common Forensic DNA Analysis Terms and Acronyms) for a comprehensive list of abbreviations.

9PLEX: Test code used for submission of samples for Profiler Plus testing XPLEX: Test code used for submission of samples for PowerPlex 21, PowerPlex Fusion or Globalfiler

Dual analysis: The term used for the examination of an exhibit by two or more forensic sections (e.g. Forensic DNA Analysis and Chemistry).

#### 4 General Principles

#### 4.1 Anti-contamination procedures

QIS document 22857 (Anti-contamination Procedure) describes the anti-contamination procedures for the examination of items, which must be adhered to at all times.

#### 4.2 Continuity

Continuity is the ability to demonstrate and account for the movements and ownership of an item, meaning that at any point between when the exhibit is seized through to when the exhibit is produced in court or destroyed, its location and all persons who have come in contact with the exhibit can be determined. This provides evidence that the exhibit has not had the opportunity to be tampered with, or has not come in direct contact with other exhibits. Refer to QIS document 14077 (FSS- Legal Analysis).

When moving an exhibit or case file the physical movement must be recorded electronically in AUSLAB using the transfer function (for exhibits or case files already with a physical location) or using the add or fill functions (for exhibits or case files without a current physical location).

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In addition to recording the physical location of exhibits and case files, continuity also includes:

- Recording exhibit packaging details, including seals.
- Examination notes
- Use unique identifying numbers or barcodes for exhibits and sub-samples.
- Maintaining custody and security of exhibits at all times. Only items which are drying should be left in the laboratory overnight. All other items must be returned to the exhibit room or freezer.

**NOTE:** Staff with three initials must use three initials at all times when signing, so as to distinguish staff. Refer to QIS document 17088 (Procedure for recording handwriting specimens in Forensic DNA Analysis).

#### 4.3 Forensic Relationship

The Forensic Relationship field is provided by QPS to indicate the relationship between the exhibit and the case, and where the exhibit appears to have originated from. An exhibit may have one or more forensic relationships assigned to it.

The Forensic Relationship of an exhibit can be viewed in two ways:

- In the SF9 Summary Page of a case, listed in the "Relation" column.
- On the EXR/EXH page the exhibit, listed in the "For Relationship" field.

#### Definitions:

- N: No further work (All work must be ceased for items with this forensic relationship)
- S: Item/sample is believed to have originated from the suspect
- V: Item/sample is believed to have originated from the victim
- E: Item/sample is from a known source, to be used as an elimination sample
- X: Item/sample is has been found/originated from the point of entry/exit
- W: Item/sample is believed to have come from/been used as a weapon
- A: This item sample has been identified as a key sample of interest and is preferred to be sampled due to admission/ intelligence value

#### 4.4 Priority

The QPS will designate a priority for a case and for exhibits (which may be different). A case/sample may be given the following priorities:

- Priority 1 (Urgent): Samples specifically approved by the QPS for processing in 3-5 day turn around. Samples may only be processed as Priority 1 with the approval of the Senior Scientist, Team Leader or Managing Scientist.
   Samples identified as needing to be processed before routine samples, due to an identified specific issue e.g. pending court date for case
- Priority 2 (High): Allocated based on crime code and generally used for crimes against a person.
- Priority 3 (Medium): Allocated based on crime code and generally used for crimes not against a person (i.e. property crime).
- Priority 6 (Cease work): Used to designate that a sample no longer requires processing and all work is to be ceased.



Priority 4 and Priority 5 have been retained for legacy samples from old cases that
are yet to be processed. If any of these samples are identified as requiring
processing, they are to be changed to an appropriate higher priority based on the
case type and other specific information to the case.

The priority of a sample/case may change at any stage and should be reviewed when determining testing or re-testing requirements.

#### 4.5 Clinical notes

The QPS can enter examination strategies, or other information to guide the examination by Forensic DNA Analysis in Exhibit Notes field in the Forensic Register, which electronically populates the Clinical Notes field in AUSLAB.

#### 4.6 Dual Analysis

Dual analyses must be completed in the Evidence Recovery laboratory as this location has the optimal environmental conditions for DNA sampling.

Exhibits which are to be transferred to the custody of Forensic DNA Analysis must be receipted as per normal receipting arrangements through the Property Point. Where the item is not transferred to Forensic DNA Analysis, but is maintained in the custody of another section (e.g. when samples are suspected of containing prohibited substances), this is documented in the UR notes.

Where the dual analysis involves hazardous chemicals or other substances (i.e. drugs, explosives etc) the relevant forensic section is responsible for making a hazard assessment and documenting this in the UR notes for that case. This assessment must include personal risk to staff during examination, storage and subsequent analysis as well as potential risks to equipment.

#### 4.7 Managing Worklists

Evidence Recovery is responsible for managing four generic worklists: SAWL, 1BT, ESMP and SALIVA. To access these worklists from AUSLAB main menu:

- Press 5. workflow management
- Press 1, workflow menu 1
- 3. Press 1, workflow lists
- 4. Highlight required list, e.g. SAWL, SALIVA, 1BT and press enter

#### 4.7.1 SAWL list

SSLU are responsible for adding cases to this list which involve a sexual element to the offence. Management of the SAWL list is a rostered task. The rostered scientist is responsible for reviewing this list on a daily basis and actioning entries as required:

- Responding to UR note entries as necessary
- Compiling case files (where required) and formulating examination strategies for SAIKs (all examination strategies must be documented in the UR notes for that case and reviewed by a scientist competent to examine SAIKs).
- Where items have been delivered for sexual assault cases, the rostered scientist
  will check to see if the Forensic Relationship field has been completed for each
  item. If a Forensic Relationship has not been entered the scientist will enter a UR
  note requesting this information from the QPS, and place the case on the 1WPP list.
- Checking items received for a Yellow case (particularly in tubes) to determine testing requirements (i.e. if semen screening is required). This may require access to the Forensic Register.



#### 4.7.2 1BT list

SSLU use the 1BT list to notify the Evidence Recovery team that action is required for a particular case. The specific advice is recorded by SSLU in the UR notes and may include:

- · Testing is no longer required for one or more exhibits
- A change to the priority of a case
- Additional items have been received
- Requests to contact an QPS officer to provide advice
- Testing is to be re-started or commenced on a previously halted exhibit or case

#### 4.7.3 SALIVA list

This list contains all samples that require Phadebas supernatant testing. Refer to QIS document 17193 (Phadebas Test for Saliva) for further details.

#### 4.7.4 ESMP list

This list contains reference samples which require manual sampling by an ER scientist. It is important to check whether these samples do require processing, i.e. if a duplicate FTA sample has been received.

#### 5 Pre-examination preparation

Before commencing the examination of an item all available case details should be reviewed to determine the type of examination and testing which is required. This information may also be used to prioritise examinations. The following items should be reviewed:

- UR, Specimen and Clinical notes
- Medical notes including SAIK paperwork
- QP127 (if available)
- Forensic Relationship
- Exhibit description

Where the above information does not provide sufficient information to determine testing requirements the following additional strategies may be employed:

- Accessing the Forensic Register (HP5 only)
- Contacting the Investigating Officer, SOCO or Scientific Officer either directly or through SSLU via AUSLAB (1WPP list)
- Contacting the QPS DNA Sample Management Unit
- Contacting FMOs or FNEs

All communications must be recorded electronically in AUSLAB using UR Notes.

**Note:** Specific details relating to the examination of sexual cases are outlined in QIS document 32106 (Examination of Sexual Cases)

#### 5.1 Case File Documentation

QIS document 17117 (Procedure for Case Management) describes when a paper case file is required to be created and the process for creating a case file.

When making handwritten examination notes, including making sketches and annotating images, the following general principles must be followed:



- Only approved examination forms (located in QIS) can be used for making examination notes. Printed copies of these forms are kept in the Evidence Recovery laboratory.
- All notes must be legible and in pen.
- All sketches and diagrams must be in pen; however areas of interest and staining may be in coloured pencil.
- Errors must be crossed out once (so that the original item is still visible) and initialled and dated.
- Examination notes must be made contemporaneously and in the examination area.
- Registration of samples must be completed as soon as practicable after the examination.

#### 5.2 Amended receipts

Amended receipts are used where there is a discrepancy between the original receipt and the actual contents of the exhibit/s. QIS document 26040 (Procedure for Issuing Amended Receipts in the Forensic Sciences Property Point) describes the process for issuing amended receipts.

#### 5.3 FERROs (Forensic non-compliance feedback)

A FERRO should be created when a submission for Forensic DNA Analysis does not conform to our standard operating procedures and is not described in an EXH line.

To create a FERRO, From EXH page:

- 1. Press Shift F10 Registration
- 2. Press End to move cursor to test code box
- 3. Enter FERRO in box, press enter
- 4. F7, F4, F4 (automatically returns to EXH page)
- 5. Pg down to forensic non compliance feedback page
- 6. Press Tab to contact person
- 7. Press Shift F2 bulk edit and enter 'dnaer'. Press F4 to save
- 8. Enter barcode(s). Press F4 to save
- 9. Enter reason. Press F4 to save
- 10. Enter comments as per standard phrasing (see SOP 26071). Press F4 to save
- 11. Press F8 to escape from bulk edit
- 12. Press Shift F12 and enter 'FERRO' to add to list

The Senior Scientist is responsible for reviewing and sending FERROs to the QPS.

#### 5.4 Exhibit Delivery

The Property Point deliver exhibits to Forensic DNA Analysis typically once each normal work day (usually in the afternoon). Exhibits are tracked to the Exhibit Room Shelves (FBEXS696-700) or DNA Freezer Shelves (FBDFS1-4). Exhibits may also be stored in other locations within the Exhibit Room or Freezer and must be tracked to their physical shelf location in AUSLAB.

#### 6 Examination

Exhibits must be retrieved from their storage location and tracked to the Evidence Recovery laboratory (DAER1).



#### 6.1 Print receipt and labels

The receipt must be printed and attached to the examination notes. To view receipt- press INSERT on the correct casefile page

#### To print receipts:

- From the receipt page SF11
- F7 Direct to
- Enter printer name

#### The following labels are printed:

- · Receipt label to attach to each examination page
- Exhibit barcode (FBLAB6) for examination notes and labelling exhibit as necessary
- Exhibit barcode (FSAMP) to label sample submission tubes

#### To print labels:

- 1. From AUSLAB main menu press 7, 1, 3
- Type in label type wanted e.g. FBLAB6 or FSAMP
- 3. Change 'Printer Name' as required e.g. fblabel2
- 4. Press F6, F5
- 5. Scan barcode/s required
- Press Esc from edit mode
- 7. Press F7 to print

#### 6.2 Description of packaging

Using QIS documents 17033 (General Examination Record (Unruled)), 17034 (General Examination Record (Ruled)) or 22870 (Forensic DNA Analysis Outer Packaging Record) describe the packing of the exhibit, working from the outer packaging to the inner packaging.

The following minimum details must be recorded for each layer of packaging:

- Packaging type (e.g. HSPB, CSPB, BPB)
- Seal type, whether the seals are intact and if they are signed and/or dated.
- A brief description of the labelling including unique identifiers (typically barcode).
- QPS outer packaging and all other packaging should be photographed.
- HSPB outer packaging which has been created by Property Point (e.g. for SAIKs) does not need to be photographed.

Packaging should be opened in such a way as to maintain the original seals. Where packaging has been opened by it must be signed and dated.

#### 6.3 Digital Imaging

Images must be taken for exhibits which are complex and/or difficult to accurately describe in written notes. Smaller, uniform items (i.e. cigarette butts, fingernails, straws etc) do not require images, except where there is unusual staining, damage or other features which are difficult to describe.

A scale and exhibit barcode must be included in every image. QIS document 20080 (Digital Imaging of exhibits in Forensic DNA Analysis) describes the digital imaging process in more detail, including the use of cameras, uploading images to AUSLAB and annotating images.



#### 6.4 Exhibit Numbering

Each exhibit must have a unique barcode registered in AUSLAB, which is used as the common identifier between Forensic DNA Analysis and the QPS. Forensic DNA Analysis assigns a secondary identifier to each exhibit which is a reference to the receipt for that exhibit. Each exhibit is assigned a secondary identifier according to the following format: 123456789-001, where 12345789 is the receipt barcode and each exhibit is assigned an ascending number (i.e. -001, -002, -003 etc).

Subsamples are assigned numbers according to the following example: The first subsample from exhibit 123456789-001 is assigned the secondary identifier 123456789-001-1; the second subsample from the same exhibit is then assigned the secondary identifier 123456789-001-2.

#### 6.5 Description of item

Exhibits must be described according to the following minimum requirements:

- What it is
- Size
- Labelling/brand
- Colour
- Staining (including any presumptive tests conducted)
- Physical appearance of damage (without commenting on the cause of the damage)

Staining must be further described using according to its:

- Shape
- Distribution
- Colour
- Size (including measurements)
- Intensity
- Which side of the item the stain may have originated from
- Any presumptive tests performed
- Odour if applicable

Please note that images can be used to describe the physical appearance of stains.

Extraneous surface material such as hairs, glass fragments, fibres and vegetative matter may easily be lost from an item. These materials should be noted in the examination notes, collected into CSPBs (labelled with item barcode) and kept with the repackaged exhibit.

#### 6.6 Presumptive or Screening tests

Forensic DNA Analysis uses the following screening tests:

- TMB test for blood see QIS document 17190 (Tetramethylbenzidine Screening Test for Blood)
- AP test for seminal fluid see QIS document 17186 (The Acid Phosphatase Screening Test for Seminal Stains)
- Phadebas test for saliva (paper and supernatant) see QIS document 17193 (Phadebas Test For Saliva)
- P30 test for seminal fluid see QIS document 17185 (Detection of Azoospermic Semen in Casework Samples)
- Microscopy for spermatozoa see QIS document 17189 (Examination For & Of Spermatozoa)



The results of all presumptive testing, including positive and negative control results, must be recorded in the examination notes. Areas on the exhibit which test positive for a presumptive and/or screening test can be circled using a chinagraph pencil or felt tip pen.

Where an examining scientist elects not to perform a presumptive or screening test a justification must be recorded in the Specimen Notes for that exhibit (e.g. that presumptive testing would consume the sample). Where an examination strategy has not been prepared, the examining scientist is responsible for assessing the exhibit and selecting the appropriate presumptive and/or screening tests as per Pre-examination preparation above.

#### 6.6.1 Phadebas supernatant testing

Phadebas supernatant testing can be performed following submission of sample for DNA Analysis. This possible screening strategy should be taken into consideration when deciding whether Phadebas screening prior to DNA Analysis will consume the evidence.

Items which are legitimately expected to contain saliva and the presence of saliva is not probative (i.e. cigarette butts, straws, drinking containers) do not require Phadebas testing.

When registering a sample for supernatant testing, the sample must be registered with the Specimen Type 'CSUP' and with "retain s'natant" in the 9PLEX/XPLEX processing comment, and inserted to the SALIVA list.

#### 6.7 Sample Selection

The case history, presumptive/screening test results and the staining present on the item are all used to determine which samples, and how many samples are to be submitted. The following elements should be considered when selecting samples for submission:

- Case history offence type and the modus operandi
- Number of offenders if there are multiple offenders/complainants then an increased number of samples may be required to identify as many involved persons as possible.
- Presumptive/screening test results samples of each biological fluid type should be considered for submission.
- Size, location and distribution of staining

#### 6.8 Sampling techniques

Forensic DNA Analysis uses the following sampling techniques:

- Swabbing
- Tape-lifting
- Scraping
- Excision
- Submission of whole item

#### 6.8.1 Swabbing – used for non-porous surfaces

Swabs are moistened using nanopure water or 70% v/v Ethanol, used to sample the stain, and the entire swab head is submitted for analysis. In some cases a dry swab may be used following the wet swab and both swabs combined in one tube. This strategy should only be used in consultation with the Senior Scientist.

#### 6.8.2 Tape-lifting – used for porous surfaces

The sticky surface of commercial tape is pressed against the item until the tape's adhesive properties are exhausted. Always ensure that a newly exposed section of the tape is used



to reduce the chance of contamination. The tape must be rolled with adhesive side in the middle and then placed into a tube for analysis.

#### 6.8.3 Scraping

This method is used for fabrics or surfaces where tape-lifting or swabbing are not appropriate and the stain is too large to excise. A scalpel blade (in holder), is used to scrape the top layer of the exhibit, from which a suspension is made or which is submitted for analysis directly.

#### 6.8.4 Excision

This method is used for stains/samples which are small enough to fit into a sample submission tube.

#### 6.8.5 Submission of whole item

This method is used where the entire item is small enough to fit into a 1.5 or 2ml eppendorf tube.

#### 6.9 Specific examination strategies

#### 6.9.1 Examination of clothing / footwear for epithelial cells

Generally only a small number of epithelial cells are deposited by touching or wearing items. It is best to use one side of a swab or a piece of tape no more than 2cm long to collect for submission, so as to concentrate cellular material into one sample.

High friction areas, including armpits, collars, inside collarbone, waist bands, hat bands and other parts of clothing that are in constant contact with the wearer are good areas to sample.

#### 6.9.2 Syringes

Specific Syringe Handling Kits are available to make this process as safe as possible. Refer to QIS document 17135 (Handling and Sampling of Syringes and Needles).

#### 6.9.3 Swabs

Record the amount of the swab that is stained, the colour, the stain intensity, the result of any screening tests and the amount of the swab that is submitted for DNA analysis. The entire swab head can be cut off and submitted for testing. A specific form is available for recording: QIS document 22846 (General Swab Exam Record).

#### 6.9.4 Cigarette Butts

When examining cigarette butts, indicate whether or not the cigarette appears to have been smoked, whether it has been stubbed/flattened and any brand names visible on the butt. A specific form is available for recording: QIS document 23014 (Cigarette Butt General Examination Record). When sampling cigarette butts, any tobacco and/or filters are removed during sampling and not submitted for testing.

Smoked manufactured cigarettes: Excise a 0.5cm circumference of the filter paper from the butt using a scalpel blade and submit for testing.

Smoked hand rolled cigarettes: Submit entire cigarette paper for testing.



Unsmoked manufactured and hand-rolled cigarettes: Submit the entire cigarette paper and filter paper for testing. If there is too much substrate for one tube, the sample must be submitted for extraction in multiple tubes and then pooled post extraction (see Appendix 2: Pooling).

For all cigarette butts, once sampling has been completed, any remaining portion of the item (excluding filters and tobacco) are retained in the item retention box, for further testing if required. If the examining scientist believes that a substance other than tobacco is contained with cigarette, this must also be retained in the item retention box.

Where a CSSE contains multiple cigarette butts, and not all cigarette butts have been submitted for analysis, the Sample Info 1 fields are used to communicate to the QPS the total number of cigarette butts contained in the CSSE and the number of cigarettes which have been tested. To do this, complete the Sample Info 1 fields as per below:

- For the Parent exhibit add to the end of the existing item description add 'N total cig butts' where N is the total number of cigarette butts in the CSSE.
- For each cigarette butt submitted for tests add 'cig butt 1 of N', 'cig butt 2 of N' etc.

#### 6.9.5 Fingernails

Fingernails or fingernail scrapings are examined to find blood or cells on or under the nails. Clippings, loose scrapings, scrapings on swab sticks or complete nails may be submitted. Describe the fingernails in terms of number, size and any visible staining. Submit these items for analysis (noting if all or some submitted). Generally samples for each hand are pooled, i.e. all samples from the left hand together and all samples from the right hand together.

Samples are not pooled where the case circumstances require:

- seeing which finger was used in digital penetration
- where the items are whole swabs
- where there are TMB positive and TMB negative samples.

#### 6.9.6 Post Mortem samples

The examining scientist is responsible for assigning an EXH barcode to the PM samples as a whole, which must be communicated to the Senior Scientist who will forward this information to DRMU via QPS email. The receipt under which the samples are submitted usually has an associated Coronial case number. Before any subsamples are registered this Coronial case number needs to be changed to the associated QP number by an AUSLAB Corrections Officer. If subsamples are registered under a Coronial case number the EXH lines will not be transmitted to QPS.

PM samples may include sexual assault swabs and/or slides (high vaginal, low vaginal, vulval etc), body swabs, pubic hair, head hair, fingernail clippings or scrapings.

Intimate swabs (i.e. those taken for a sexual offence including vaginal, anal and oral) are examined according to standard SAIK examination procedures. However, semen negative intimate PM swabs are all submitted for analysis - refer to QIS document 32106 (Examination of Sexual Cases).

Sometimes the fingernail clippings include a portion of tissue or part of the finger. In this case a moistened swab can be used to sample potential foreign DNA from the underside of the nail, taking care not to sample the deceased person's tissue (i.e. targeting the distal end of the nail).



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#### 6.9.7 Heavily stained items and known infectious items (universal precautions)

Heavily stained or soiled items, or items containing known infectious material may be examined in the fume hood to prevent contamination and infection. Double gloving should be considered during examination, as this provides an additional physical barrier between the examiner and the item.

#### 7 Sample registration

#### 7.1 CS page

The case status must be updated:

- If the status is 'Started' no change is required
- If the status is 'Received' it must be changed to "Allocated' then to 'Started'
- If the status is "Allocated" then change to "Started"
- If the status is 'Report Issued', or 'Analysed- Report not Required', change the status to 'Reactivated'
- If the status is 'Reactivated', 'Sent to Peer Review' or 'Return from Peer Review', no change is required

Ensure that the Team is entered on the CS page and also in the BTEAMS field on the registration (SF10) page. The Priority listed on the CS page should be used as the priority for all samples submitted for that case (unless otherwise specified in the UR notes).

#### 7.2 EXH entry

An appropriate EXH line (or multiple lines) must be entered on the EXH page for each exhibit. QIS document 23008 (Explanations of EXR/EXH Results) provides a list of all EXHs and their expanded wording.

Enter the appropriate barcode for the EXH line, which may be the exhibit barcode (where the result refers to the entire item, or the entire item is submitted for testing), or a subsample barcode. There is no need to enter duplicate EXH lines where multiple subsamples are submitted for the same analysis.

To enter an EXH line:

- 1. Press 3 Patient Enquiry
- 2. Scan exhibit barcode
- Press Shift F2 to edit the page
- 4. Fill in appropriate EXH lines with barcodes and results/status (Use F1 look-up)
- 5. Fill in team name (if not already populated)
- 6. Press F8 to exit edit mode

#### 7.3 Registration of exhibits

Sample registration, from EXH page:

- 1. Press Shift F10 Registration and complete the following fields:
- Specimen type e.g. EXHIBITS (if there are sub-samples) or FSS
- Primary site e.g. SWAB, CIG BUTT
- 4. Client Ref (receipt # 00x)
- DNA priority
- 6. Sample Info 1 description of the sample, any relevant barcodes and micro result if applicable
- 7. Enter 'FBX' in box, press enter and follow prompts at bottom of page



- 8. Enter '9PLEX/XPLEX' in box, press enter (for swabs, cig butts and items consumed by sampling) A prompt will appear enter in any necessary processing comments, e.g. ~150ul nano H20 added.
- 9. Press F7, F4, F4 (automatically returns to EXH page)
- 10. SHIFT F9 to view summary page

Note: If the item is a piece of clothing, item ownership can greatly assist with DNA profile interpretation. If there is no indication within the item details / descriptions transferred from the Forensic Register or on the item packaging as to whom the item of clothing is attributed to (or if is unknown), including information within the Forensic Relationship field (or if this information is not informative e.g. when there is more than one victim or suspect), list insert the exhibit barcode onto the Item Ownership List "ELF" for SSLU to follow-up with QPS. If there are no reference samples associated with the case at the time of examination, this is not necessary.

Commonly used specimen types include:

- HAIR or HDNA: for samples that have been identified as human hair suitable for DNA analysis.
- SFRAC/EFRAC: for samples that have semen present.
- CSUP: for samples that are to have their supernatants retained for phadebas amylase activity testing. Enter processing comment "retain s'natant". Sample should then be placed on SALIVA communication list.
- NUCT: for tissue samples

Repeat procedure for other exhibits/ samples if required.

#### 7.4 Registration of sub-samples

Sub-sample Registration

- 1. From EXH page:
- 2. Press Shift F10 Registration
- 3. Press F7, F4, Shift F5 to copy page
- 4. Scan in new barcode for sub-sample and complete the following fields:
- Specimen type e.g. FSS
- Primary site e.g. SWAB
- 7. Client Ref (receipt # 00x-y)
- 8. DNA priority
- 9. Sample Info 1 description of the sample, barcode of exhibit the sub-sample is taken from
- 10. Enter '9PLEX/XPLEX' in box, press enter. A prompt will appear enter in any necessary processing comments e.g. :retain s'natant"
- 11. F7, F4, F4 (automatically returns to EXH page)
- 12. SHIFT F9 to view sub-samples on summary page
- 13. Enter on sub-sample and add 'Team' name if not already present

Repeat for other sub-samples if necessary.

#### 7.5 Consumables and reagents

For each tube that is used, details of the tube lot number must be recorded in the audit trail for that sample barcode. Reagent details must be attached to all samples where a reagent has been used (e.g. when TMB screening performed) with an EXH page – in the case of an item with multiple sub-samples the reagents only need to be logged against the item EXH.

- 1. From the EXH page or from the 9PLEX/XPLEX page for sub-samples
- Press F12



- Press F5 consumables
- 4. Press F5 Add Entry
- 5. Use F1 look-up list and arrows to highlight required consumable or reagent
- Press Enter
- Press F6 Add Notes to record bag number or details of box number e.g. P30 kits
- Press F4 to save
- 9. Press ESC only when you are ready to escape and the reagent has been logged.

To see that the items have been assigned, from the EXH screen, press F12, F5

#### 7.6 Sample tracking

When registration is complete samples must be tracked to an ERT transfer box and placed in the transfer hatch. Transfer boxes not collected and transferred to Analytical must be stored at close of business to the Exhibit Room (FBEXS700) or if the samples are wet to the Freezer (FBDFS1).

To Store sample tubes:

- 1. From main menu
- 2. Press 2 sample processing
- Press 6 sample storage
- 4. Scan the barcode of the storage box
- Press Shift F5 to fill the rack
- Scan the barcode on the tube and place tube in rack position as indicated on the screen.

#### 7.7 Exhibit repackaging and return

Exhibits should be repackaged in the same packaging if practical. Re-seal the openings with evidence tape, sticky tape or heat seal and initial and date the seal.

If an exhibit is wet as the result of examination, it can be placed on the drying rails overnight to allow it to dry. Ensure that the rails are cleaned with bleach and ethanol before and after drying. Exhibits must have a piece of brown paper between the rail and the item, and an additional piece of brown paper covering the item.

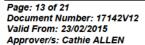
If examination of an exhibit is not complete, the item can be tracked back to the freezer or the exhibit room and retrieved at a later date. Where the examination is complete the exhibit must be tracked to the Exhibit Room return location (FBEXR1) or the Freezer returns location (FBEFR1).

To transfer an Exhibit, from the EXH page:

- Press Shift F9 summary page
- 2. Press Shift F5 to show storage locations
- Highlight line for receipt/exhibit barcode of interest
- Press Shift F7 to transfer item
- 5. Enter storage location where you want to move it to and press Enter
- You will be prompted to confirm transfer, Press Y, and Enter
- 7. Check item of interest has been transferred to desired location

#### 7.8 Tracking case files and examination notes for review

After examinations and registrations are complete, all notes are to be tracked to the DAPR1 drawer to be peer reviewed by another competent scientist and the EXH lines reviewed and validated. Refer to QIS document 17117 (Procedure for Case Management) for the review of EXH lines.





Once examination notes have been reviewed they are to be filed in one of the following locations:

- If the case is paperless, track the receipt to FBSI47 for filing by the admin team.
- If you are in possession of the case file, add the notes to the file and track to the back section of the DAPR1 drawer. Case files will stay in this location until they are transferred once weekly to a case management drawer.
- If the case file is stored to another location and you have an additional receipt, add the notes to the case file and remove the location of the receipt in AUSLAB, and track the receipt to the Case Management drawer (FBCM24).

#### 8 Examination and sampling of reference samples

All samples that are designated as reference samples **must** be examined and sampled on **Examination Bench 15**.

8.1 Examination of reference samples other than FTA cards

The principles of examination and sampling of reference samples other than FTA cards (e.g. swabs, hairs) are the same as those for casework exhibits. Refer to Examination above. The specific registration of reference samples is different refer to Appendix 3: Reference sample registration for the registration of reference samples.

8.2 Examination of FTA reference samples

For reference FTA cards, determine if the card is being sampled due to either:

- a) the unavailability of the BSD instrument for extraction preparation or if the process is being performed in Evidence Recovery due the urgent nature of a case. Or,
- b) due to failure to gain sufficient DNA from routine extraction preparation processes, and additional sample is required to yield a DNA profile.
- 8.2.1 For FTA cards being sampled for routine extraction preparation when BSD instrument is unavailable.

For these samples, an EREF test code should be visible in the SF7 Results History table. There should be no results against the EREF test code, the specimen notes should state something similar to "to be sampled by Evidence Recovery". Additionally, within the sample audit trail, the EREF test code should be outstanding on an "R21EXT" batch. If the sample has been extracted previously, a connected barcode may be registered, specimen notes will indicate under which barcode the FTA card is to be sampled. If it is unclear which barcode is to be used, or if a connected barcode is required, do not proceed with sampling and consult the Senior Scientist

- 1. Excise an approximately 5 mm x 5 mm section from each of the black circles on the FTA card and place into an appropriately labelled 2 mL screw capped tube.
- 2. Register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.

**Note:** If the FBEXAM and specimen note are to be recorded against the barcode



that the sample is being processed under. This may be a connected barcode.

- Notify the Quality and Projects senior scientist, the Operational Officer supervisor or the Analytical Team senior scientist that sampling is complete and that the sample is ready to for DNA extraction.
- 4. Return samples to the storage location that they came from with a note stating that sampling is complete.
- 8.2.2 Blood FTA cards being sampled for the first time
  - 1. Excise an approximately 5 mm x 5 mm section from each of the black circles on the FTA card and place into an appropriately labelled 2 mL screw capped tube.
  - 2. Register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.

**Note:** If the FBEXAM and specimen note are to be recorded against the barcode that the sample is being processed under. This may be a connected barcode.

- Access the SF7 results history table, order an EREF test code using the SF8 add rework function.
- 4. Return samples to the storage location that they came from with a note stating that sampling is complete.
- 8.2.3 For FTA cards being sampled due to insufficient DNA from previous extraction processing. These samples will be being processed as a final attempt to gain a DNA profile after routine methods have failed. As such, as much remaining sample as is reasonable is to be sampled for profiling.
  - Determine whether a connected barcode has been registered for this sampling process. For the registration of connected barcodes for reference samples refer to QIS document 17117 (Procedure for Case Management).
  - Excise as much sample as is feasible and place in an appropriately labelled 2 mL screw capped tube.
  - 3. Under the connected barcode, register and complete an FBEXAM test code for the sample and add an additional specimen note indicating the card has been sampled by the Evidence Recovery team. If multiple FTA samples are to be processed, registration of each individual sample is required before moving onto the next one. In this way, the FBEXAM registration time will accurately reflect the time of sampling.
  - Notify the Quality and Projects senior scientist, the Operational Officer supervisor or the Analytical Team senior scientist that sampling is complete and that the sample is ready to for DNA extraction.



Return samples to the storage location that they came from with a note stating that sampling is complete.

#### 9 Associated Documentation

QIS: 16004 - AUSLAB Users Manual - Forensic DNA Analysis

QIS: 17033 – General Examination Record (Unruled)

QIS: 17034 - General Examination Record (Ruled)

QIS: 17117 - Procedure for Case Management

QIS: 17119 - Procedure for Release of Results

QIS: 17135 - Handling and Sampling of Syringes and Needles

QIS: 17140 - Procedure for the Identification and Examination of Hairs

QIS: 17185 - Detection of Azoospermic Semen in Casework Samples

QIS: 17186 - The acid Phosphatase Screening Test for Seminal Stains

QIS: 17189 - Examination For & Of Spermatozoa

QIS: 17190 - Tetramethylbenzidine Screening Test for Blood

QIS: 17193 - Phadebas Test for Saliva

QIS: 20080 - Digital Imaging in DNA Analysis

QIS: 22846 - General Swab Exam Record

QIS: 22857 - Anti-contamination Procedure

QIS: 22870 - Forensic DNA Analysis Outer Packaging Record

QIS: 23008 - Explanations of EXR/EXHs

QIS: 23014 - Cigarette Butt General Examination Record

QIS: 23055 - General Examination Record

QIS: 23849 - Common Forensic DNA Analysis Terms and Acronyms

QIS: 23898 - SAIK Details Record

QIS: 26071 - Examination of In-tube samples

QIS: 31286 - SAIK form no semen testing

QIS: 32106 - Examination of Sexual Cases

QIS: 32639 - General Examination Form (Packaging) with microscopy

QIS: 32640 – General Examination Form with microscopy

#### 10 Amendment History

Revision	Date	Author/s	Amendments
0	17 Feb		
	1999		
1	26 Jun	V lentile	





	2001		
2	18 Sep 2002	V lentile	Amendments to references, (8) Characterisation of Biological material and (22) Reference Samples
3	26 May 2005	K Lee	Entire document revised and rewritten
4	21 Oct 2005	M Gardam	Added when to use "what appears to be" for when a confirmatory test is not available.
5	22 June 2006	M Gardam	Added Techniques for various exhibits, added AUSLAB Flowcharts.
6	14 Feb 2007	L Weston	Update with new processes for AUSLAB-LIMS
7	14 Jan 2009	J. Connell J. Munoz	Added: size references for DNA extractions; destruction requirements; dual examinations; swab and tape lift brands used; associated documents; table of contents. Removed appendix & reference to volume flowcharts. Updated processes for: off deck lysis; registration of multiple items. Separated General Examination Procedure into subheadings. Transferred information for case file compilation and AUSLAB procedures from Case Management SOP. Transferred into new template. Changed EXRs to EXR/EXHs.
QIS <sup>2</sup> Editio			T
Version	Date	Updated By	Amendments
9	25 August 2009	K.Scott	Updated requirements as per NATA assessment DEC 2008. Updated FIRMU contact emails. Addition of examination/registration of a reference sample – including appendix 6 Add submission of wet/dry swabs from skin or other items. Add submission of TMB pos. TMB neg fingernail scrapings Add N=No further work in Foren.Rel. Field. Add references to SOP 26071 Clarify photography requirements Update examination of condoms section
10	October 2013	L. Ryan A Houlding J Seymour-Murray	Split Examination of Items SOP in sexual and non-sexual SOPs. Rework entire SOP content
11	6th	J Seymour-Murray	Changed DNA Analysis to Forensic

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	December 2013		DNA Analysis, added XPLEX, updated Appendix 3.
12	15th January 2015	J Seymour-Murray A McNevin	Added minor corrections, included an updated section on reference samples, updated to new template. New multiple items screen shot. Combined 11.3.4 & 11.3.5 into 11.3.3

#### 11 Appendices

#### 11.1 Appendix 1: Registration of multiple items

Where QPS submit multiple items under the one barcode, each item can be individually assigned a barcode and an EXH ordered, so that each item can be reported separately to the QPS. The screen shot below shows a parent item which contains three items (white shirt, red shorts and black hat). The EXH has been entered so that each of these three items can be registered under the Linked No. barcode, and an EXH ordered for each of these items.



Figure 1: EXR/EXH page for original barcode



#### 11.2 Appendix 2: Pooling

The example below is based on the following examination scenario:

One parent item, an unsmoked cigarette butt (lab# PARENT), is to be sampled into two subsamples: Subsample A (lab# SSA) and Subsample B (lab# SSB). These two subsamples will then be pooled into one sample: Pooled (lab# POOLED).

PARENT barcode is the existing EXH barcode which has been transferred from the Forensic Register. SSA, SSB and POOLED are all new barcodes which must be registered by the examining scientist using standard item registration methods.

Actions for Parent Item – Unsmoked Cigarette Butt (lab# PARENT) Registration:

Client ref: -001

Test Codes: EXH, FBEXAM

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Leave as transferred from Forensic Register

#### EXH:

 EXH line: submitted results pending, using lab# POOLED as this will be the reported lab#

Actions for Subsample A (lab# SSA)

#### Registration:

Client ref: -001-1

Test Codes: 9PLEX/XPLEX, POOLED

Processing Comment: Ext & Hold

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Parent Item description; Sub Sample A

9PLEX Page only (these fields are not relevant for XPLEX pages):

Accepted Barcode: #POOLED

Connected Barcodes: #SSB, #POOLED

#### Pooled Page:

- This lab number has been pooled with Lab Number: #SSB
- Processed Using Lab Number: #POOLED
- Reported Under Lab Number: #POOLED

Actions for Subsample B (lab# SSB)

#### Registration:

Client ref: -001-2

Test Codes: 9PLEX/XPLEX, POOLED

Processing Comment: Ext & Hold

Specimen Type: FSSPrimary Site: CBUTT

Sample Info 1: Parent Item description; Sub Sample B

9PLEX Page only (these fields are not relevant for XPLEX pages):

Accepted Barcode: #POOLED



• Connected Barcode: #SSA, #POOLED

#### Pooled Page:

- This lab number has been pooled with Lab Number: #SSA
- Processed Using Lab Number: #POOLED
- Reported Under Lab Number: #POOLED

#### Actions for Pooled (Lab# POOLED)

#### Registration:

- Client ref: -001-3
- Test Codes: 9PLEX/XPLEX
- · Processing Comment: nil
- Specimen Type: POOLED
- Primary Site: leave blank
- Sample Info 1: SSA, SSB, Parent Item description in Sample Info 1

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#### 11.3 Appendix 3: Reference sample registration

#### 11.3.1 Registration of reference tissue from a coronial

Take the following steps to register the sample:

- Locate the receipt page for the coronial samples
- 2. Access the registration page for the receipt (Shift F10)
- Copy the page (F7, F4, Shift F5)
- 4. Scan the new barcode for the reference tissue
- Specimen Type: Nucleospin-Cells-Ref (NUCCR)
- 6. Primary Site: leave blank
- 7. Client Ref: receipt barcode
- 8. Sample Info 1: enter a description of the tissue (e.g. liver)
- Test Codes: FBEXAM, REF21
- 10. Save the registration (F7, F4, F4)

#### 11.3.2 For Blood Swab/ Blood Cloth

Specimen Type: Reference Blood

Test Code: REF21

#### 11.3.3 For Cell Swab/ Reference Hair (for samples in ESMP box)

- 1. Note down the delivery person and receiving person details
- 2. Enter into the registration page (SF10)
- Add FTAR test code
- Delete REF21 & FTAREF test codes
- Change specimen type to RCELLS/RHAIR
- 6. Save registration (F7, F4, F4)
- Re-enter the SF10 registration page
- Delete FTAR test code
- 9. Add FTAREF & REF21 test codes
- 10. F7,F4,F4 to save
- Check audit trail, it should be on a RFIQMAX batch.



AR5

#### **Amanda Reeves**

From:

Amanda Reeves

Sent:

Wednesday, 11 May 2016 2:46 PM

To:

Justin Howes (.

Kylie Rika

Subject:

FW: Diff lysis slide investigation

Hiya

I would like to follow this email with a quick conversation if possible please.

ta

#### Amanda Reeves Dip Mgt BSc MSc For Sci

Senior Reporting Forensic Scientist Forensic DNA Analysis | Police Services Stream Forensic and Scientific Services Health Support Queensland

Department of Health I Queensland Government

# HealthSupport

Queensianu



From: Anna Lemalu

Sent: Wednesday, 11 May 2016 2:41 PM

To: Amanda Reeves

Cc: Josie Entwistle; Adrian Pippia; Penelope Taylor; Thomas Nurthen; Jacqui Wilson; Matthew Hunt

Subject: Diff lysis slide investigation

Hi Amanda,

The result of our discussion included the following suggestions:

- -Identifying a staining/fixing issue:
  - -Suggest collecting run off from slide washing, centrifuging and making a slide to stain and observe possible presence of cellular material that has washed off
  - -Is liquid added to the slide spread out to increase surface area to facilitate drying? Or just left as a drop?
  - -Suggest making sure that slides are fixed properly on the hot block prior to staining. An experiment to identify how long is sufficient should be conducted. The SOP does not clarify how long slides are left, what temperature the hot block is on, or how much sample is added these variables should be investigated for best outcome and then fixed.
- -Suggestion of agitating swab and water with pipette prior to vortexing and waving slide through flame prior to staining.
  - -Suggest having a parallel duplicated study using methanol as a slide fixative before the addition of stain.
- -Datamining (which may or may not include the examination of diff slides not done at the time) of past samples including:

- -Result of diff slide from micro neg/PSA pos samples
- -Result of diff slide from micro pos/low sperm count samples
- -Result of diff slide from high quant/ low micro samples
- -Collection of epithelial number data and sample type for these datamined samples also (with the thought that a low number of epi cells in certain samples could indicate the loss of sample)
- -Investigation of the amount of liquid added to the swabs
  - -Too little added may mean that sperm present may not be expelled from the swab
  - -Too much added may mean that the sample is too diluted
- -Suggest making control swabs and checking the process step by step

The major overarching concerns of this issue are the fact that in certain circumstances we may not have sent samples for DNA profiling at all (micro, AP and PSA neg) and therefore have missed evidence. Also, occasionally we are asked in court specifically about the number of sperm seen in a sample – if we know that this number is unreliable, how happy will reporters be to quote numbers?

Thanks,

#### Anna Lemalu BSc PGDipSci MSc (Forensic Science)

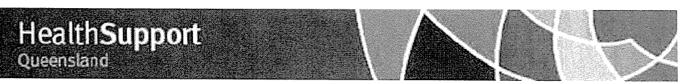
Reporting Scientist

Forensic Intelligence & Reporting | Forensic DNA Analysis | Police Services Stream | Forensic & Scientific Services

Health Support Queensland

Department of Health | Queensland Government





## Looking at old diff slides

From: Allan McNevin To: Abigail Ryan < Allan McNevin < Chelsea Janine Savage < Cindy Chang < Seymour-Murray < Margaret Brian Shannon Thompson < Sharon Byrne < Valerie Caldwell < Wed, 11 May 2016 11:39:07 +1000 Date:

Hi team,

As discussed in the meeting, we need to go back through some diff slides and see what we can see.

If you are looking for something to do, feel free to work your way through some. My thought was that we would just do a few at a time.

The spreadsheet I have started for recording everything is

G:\ForBiol\AAA Evidence Recovery\Projects and Datamining\2016 - Diff Lysis slide micro v original micro.xls

If you are thinking of doing some, please let me know.

Cheers Al

#### Allan McNevin

Forensic Scientist - Advanced, Evidence Recovery Team
Forensic DNA Analysis | Police Services Stream
Forensic and Scientific Services | Health Support Queensland
Department of Health | Queensland Government



# Health Support

Queensland Forensic and Scientific Services

## Forensic DNA Analysis - Management Team Meeting

Date: 27 May 2016 Time: 9.30am – 11.00am

Venue: FSS CR611

1. Present

Allan McNevin (ARM) Kerry-Anne Lancaster (KAL)

Cathie Allen (CJA) Luke Ryan (LBR)

Kirsten Scott (KDS) Sharon Johnstone (SMJ)
Kylie Rika (KDR) Wendy Harmer (WAH)

2. Apologies - Justin Howes (JAH), Amanda Reeves (AJR)

3. Guests - Deb Whelan (DAW)

#### Agenda items

/ igeniaa		
Item	Торіс	
1.0	Confirmation of previous minutes - SMJ	
2.0	Conflicts of Interest - Nil	
3.0	Action Register – See below	
4.0	4.1 Workplace Health & Safety Issues – OH&S will be assessing a workstation to attempt to identify any potential risks. Strut has been replaced on the centrifuge which had previously caused a minor injury.  4.2 Analytical Issues of Note - LBR Nil – other than MPIIs which are still causing issues.  4.3 AUSLAB Working Party Update – KDS Have had a few service call submissions – ie prep for QIASymphony	
	4.4 Project Updates	
	Project #152 – Y-Filer Plus – LBR.	
	ALL has prepared the written part for the mixtures and forwarded to AJR & KDR for peer review.	
	Project #168 – Validation of QIAsymphony – LBR	
	Training on the instrument has commenced and final touches to the report.	

Page: 1 of 3

Document Number: 24765T9 Valid From: 19/09/2014 Approver/s: Helen GREGG



Project #170 – Reassessment of in-house stutter thresholds and stutter file used in STRmix – JAH.

Report has been sent for peer review by management team – to be completed by 3<sup>rd</sup> of June.

Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega) – LBR

3500 part has not been progressed due to other parts of the project. Mixtures have been assessed by EJC, however still uncertain about the baseline due to the previous thresholds. Have completed the Saturation plate and have run the 10 samples used in baseline plates (have reamped at 0.6 and 0.7ng) to be included in baseline data set – to see the effect on the calculated baseline – which is currently being read by LBR. Anticipate getting this out to management team by today or Monday 30<sup>th</sup> May. The additional amps have been covered by a one page amendment document by EJC. Have 55 tubes of CC5 – which will last about 4 weeks, so this is a time critical project that needs to be progressed as a priority.

Project #TBC Hamilton Instruments being validated - LBR

2 instruments delivered today (1 to come) – pre-PCR and CE instruments – training begins in about 2 weeks. Validation project will commence after training. The 3<sup>rd</sup> instrument to be delivered in June – and will be used for Pre-PCR.

Sperm seen on Diff Lysis extraction slide vs ER suspension slide – ARM

Discussed graphs and results produced so far, all agreed that ARM would initiate a project plan for the next step.

#### Projects on-hold

Project #146 - GlobalFiler - LBR

On hold until new size standard has been completed (17.03.2016)

Project #172 - Phadebas testing from suspension in ERT - ARM

Pending outcome of project on how suspensions are made. (12/05/2016)

#### 5.0 New business

**5.1 Risk Assessments - KDS** As a result of Audit#21986 (late December 2015) - in the January Management Team meetings we allocated risk assessments to each of the teams for completion.

According to QIS there are still are number outstanding. (Open Link Doc.)
ForBiol\DNA Analysis Team Meetings\DNA Analysis Management Team\2016\Jan Jun\Risk Assessments\_2016.xls

ACTION: JAH to advise KDS the staff member assigned to each of the FRIT allocated risks.

#### 5.2 FR Go Live for Forensic Chemistry – CJA

Brief overview provided of how it went with the team.

#### 5.3 Maternity Leave Backfill of HP4 – KDR

Cassandra James will be joining FRIT on the 11<sup>th</sup> of July due to Maternity Leave of 3 staff members.

#### 6.0 New business – for noting



## **Next Meeting**

Date: 9 June 2016, 9.30am

Venue: CR611

#### **ACTION REGISTER**

Minutes Reference	Item Number	Subject	Action	Action Officer	Status
14/04/2016	5.2	Validation Baseline Methods	SMJ to complete a project initiation document	SMJ	Closed
12/05/2016	4.4	Project #171 Verification of the Internal Lane Standard from CC5 to WEN (Promega)	An email (including clarification) is to be sent out with voting options	LBR	Closed
12/05/2016	4.4	Project #172 – Phadebas testing from suspension in ERT	ARM to look at the way in which suspensions are made.	ARM	Closed
12/05/2016	5.4	Sperm seen on Diff Lysis extraction slide vs ER suspension slide	Project will be monitored by ARM and KDS.	ARM / KDS	Closed



#### **Allan McNevin**

From: Allan McNevin

**Sent:** Friday, 27 May 2016 12:23 PM

To: Deborah Whelan; Allan McNevin; Amanda Reeves; Cathie Allen; Justin Howes; Kerry-

Anne Lancaster; Kirsten Scott; Kylie Rika; Luke Ryan; Sharon Johnstone

**Subject:** FYI - Project proposal #181 - Sperm microscopy sensitivity

Hi all,

Just as an FYI – As discussed in the management team meeting today, the further investigation into the differences between ER microscopy and Diff Lysis slide microscopy will not be a formal change proposal and I am giving it proposal # 181 and calling it "Investigation into sensitivity of spermatozoa microscopy"

#### Cheers

ΑI

#### Allan McNevin

Forensic Scientist - Advanced, Evidence Recovery Team Forensic DNA Analysis | Police Services Stream Forensic and Scientific Services | Health Support Queensland Department of Health | Queensland Government

## Update on DLYS slide project

From: Kirsten Scott <

To: Justin Howes < Amanda Reeves < Co: Allan McNevin < Kylie Rika <

**Date:** Fri, 27 May 2016 10:54:38 +1000

Dear Jush and Amanda,

Quick update......

At today's management team meeng Allan presented a block of data which compared original ER slide-read data to DLYS slide read data (summarised in the spread-sheet below). I am sure Allan would be happy to discuss with you - if needed.

G:\ForBio\\AAA Evidence Recovery\Projects and Datamining\2016 comparison of original v diff micro\2016 - Diff Lysis slide micro v original micro.xlsx

As a result of the discussion at the management team meeng it was agreed that we would do what is essenally a semen serial diluon (with epi's) and look at AP, PSA and Micro results to allow us to look at sensivity/de tectability. A project proposal will be prepared over the next few weeks.

If you would like to discuss this further please see Kirsten or Allan

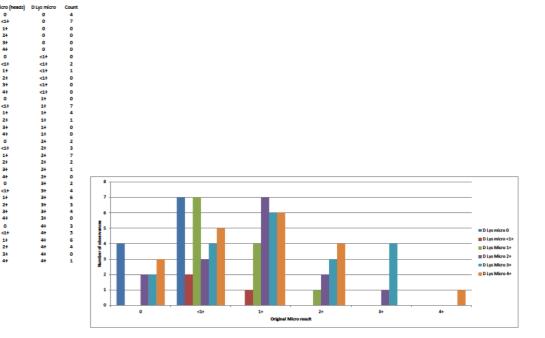
Kirsten

#### Kirsten Scott PhD BSc (Hons) DipMn GDipEd GCEd

A/Team Leader Evidence Recovery and Quality
Forensic DNA Analysis
Police Services Stream | Forensic & Scientific Services | Health Support Queensland



Client reference	tails	heads	AP D	30 Diff Lysis batch ID	diff Ivsis micro result	Siys Quant (1st run) Other notes	tails > heads						
	0	<1+		/a CWDMAX20140102 02	3+	0.0444 casefile FB2CFB93		Orig micro (heads)	D Lys micro	Count av quant	No instance where more sperm with tails was	> sperm heads on original n	micro Orig micro (head
	0	<1+	n/a r	√a CWDMAX20140102 02	0	0.0172		0 1		4 0.01005	In all instances where 1+ tails were seen, 2+ o		
	0	<1+	n/a r	√a CWDMAX20140102 02	0	0		0	<1+	O N/A	No instances of 2+ or more sperm with tails so		41
	0	<1+		n/a CWDMAX20140107_02	0	0.0147 exam notes FBCM24		0	1+	O N/A			1+
	0	<1+		/a CWDMAX20140123_01	0	0.0231 casefile FB2CFB95		0	2+	2 0.332	Number of instances Orig micro > D lys micro		2+
	0	1+		Va CWDMAX20140128_01	1+	0.00928 casefile FB2CFB93		0	3+	2 0.8861	Number of instances Orig micro = D lys micro	17	3+
	0	1+		/a CWDMAX20140128_01	2+	0.0534 exam notes FBP34		0	4+	3 0.32247	Number of instances Orig micro < D lys micro	52	4+
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	0	1+	n/a r	n/a CWDMAX20140210_01	2+	0.262		1+	4+	6 1.55558	1+ 0.09833 12	1+ 0.60497	24 4+
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	0	<1+		/a CWDMAX20140227_01	3+	0.344		2+	3+	3 0.8432	Number of instances orig micro pos, diff micro		3+
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	0	<1+	n/a r	√a CWDMAX20160202 01	4+	2.1598		4+	4+	1 20.754	2016 37		4+
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#### **Paula Brisotto**

From: Kylie Rika

Sent: Monday, 8 August 2016 10:48 AM

To: Jacqui Wilson; Adrian Pippia; Claire Gallagher; Angela Adamson; Cassandra James;

Allison Lloyd; Thomas Nurthen; Rhys Parry; Timothy Gardam; Amanda Reeves; Emma Caunt; Alicia Quartermain; Anne Finch; Emma Caunt; Hannah Pattison; Ingrid

Moeller; Josie Entwistle; Matthew Hunt; Penelope Taylor

Cc: Paula Brisotto

**Subject:** Evidence recovery - change in process

Hi all

Due to concerns and identified potential risks associated with the possibility of missing semen with current ER processes, we are making a minor change to processes effective immediately.

Please note that this change in process is being done to mitigate against the above risk, as well as buy us time to further investigate the current process and develop / test potential process improvements (Emma and Allan will be working on this over the next few weeks)

#### The change

The change is around the examination for semen / spermatozoa, and relates to all examinations including but not limited to SAIK swabs, fabric scrapings etc. There are some minor exceptions to current processes

Exception #1 Samples that are micro negative for sperm and AP negative are to be submitted for Differential Lysis extraction

Exception #2 Samples that are micro negative for sperm and AP positive, P30 negative are to be submitted for Differential Lysis extraction

#### Additional process change

All samples from exceptions #1 & #2 above, as well as any other samples that are micro negative for sperm (e.g. Micro Neg, AP pos, p30 pos) are to have the Diff Lysis slide read as a matter of course

#### Impact on EXH's used

In the instances noted above, where there are no sperm seen on microscopy and the sample is being submitted for differential lysis (and will therefore have a diff slide read), the presumptive EXH line "Submitted – results pending" will be used and then, after reading the diff slide ERT can then use any of the following lines as appropriate "Presump. PSA test positive, no sperm found"; "Semen not detected"; "Micro positive for sperm. Submitted-Results pending" or "micro neg for sperm"

Please feel free to see Emma or myself should you have any questions.

Line managers please pass onto any other persons as applicable.

**Thanks** 

Kylie

From: Kirsten Scott
To: Allan McNevin

Subject: RE: Project #181 - project plan and experimental design

Date: Tuesday, 6 September 2016 6:35:00 AM

Allan,

I am happy with the project as written

#### Kirsten

From: Allan McNevin

Sent: Thursday, 1 September 2016 12:55 PM

To: Allan McNevin; Amanda Reeves; Deborah Whelan; Justin Howes; Kirsten Scott; Kylie Rika; Luke

Ryan; Paula Brisotto; Timothy Gardam; Emma Caunt; Rhys Parry **Subject:** Project #181 - project plan and experimental design

Hello all,

Please review the following documents and complete the risk assessment in the project plan document

I:\Change Management\Proposal#181 - Sperm microscopy sensitivity
Proposal#181 Investigation of sperm micro sensitivity - Experimental design v1.0.docm
Proposal#181 Investigation of sperm micro sensitivity - Project plan v1.0.docm

I understand there is a change over of acting and when it's time to put pen to paper I will update who is in what seat, and with that in mind, please provide feedback to Emma and myself by COB next Friday

**Thanks** 

Αl

#### **Allan McNevin**

Forensic Scientist - Advanced, Evidence Recovery Team
Forensic DNA Analysis | Police Services Stream
Forensic and Scientific Services | Health Support Queensland
Department of Health | Queensland Government

## Follow up from management team meeting - project #181 next step

Allan McNevin <"/o=queensland health/ou=exchange administrative group From: (fydibohf23spdlt)/cn=recipients/cn=mcnevina"> To: Allan McNevin < Cathie Allen < Justin Howes 4 Kirsten Scott < Kylie Rika Luke Ryan < Matthew Hunt Paula Brisotto Sharon Johnstone Cc: Emma Caunt Fri, 17 Mar 2017 08:34:42 +1100 Date:

Hello all.

As per the outcome from the management team yesterday, please vote Approve / Reject on the following way forward with the next step for project #181

Proposed next step

Proceed with tesng a method of using a spin basket in ER to make slides as outlined in the previously disseminated presentation, with the exception to a tonly 1 in 50 and 1 in 100 semen dilutions will be used to test method. Also noted is that the flip-top tubes will not be suitable for Analycial so further tube transfers to be carried out

Copied from slide 6 of the presentaon:

- Proposed procedure:
  - Sample into flip-top tube (same as used for spin baskets)
  - Create suspension & make micro slide as per current
  - Transfer swab to spin basket & spin (using analyc al sengs)
  - Put spin basket contents and most of supernatant into new tubes (one each)
  - Resuspend pellet and make new slide
  - Submit pellet for diff lysis & make diff lysis slide
  - Submit ER spin basket for diff lysis & make diff lysis slide

Please reply by COB Wed next week (22/03)

Cheers

Αİ



#### Allan McNevin

Forensic Scientist - Advanced

Evidence Recovery Team, Forensic DNA Analysis, Health Support Queensland, **Department of Health** 

HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Proposal # 181 - Investigation into sensitivity of spermatozoa microscopy

Brainstorming document

#### Concern / Issue:

Negative ER microscopy, AP/p30 neg result = sample not processed through Diff Lysis (or prioritised and not processed at all) or;

Negative ER microscopy, sample from a washed item where AP/p30 unreliable = sample not processed through Diff Lysis (or prioritised and not processed at all)

Issue with not doing a diff lysis is that biological origin cannot be commented on

#### **Initial investigation**

Looked at a range of diff lysis slides for a range of samples from 2014, 2015 and 2016. Samples consisted of a small number where Reporting scientist had already requested the Diff Slide to be examined, and others where the case had been finalised or was in progress and no statement request had been received; compared results for original microscopy against results for diff lysis slide microscopy. Results in G:\ForBiol\AAA Evidence Recovery\Projects and Datamining\2016 comparison of original v diff micro

#### **Further investigation**

From management team meeting 27-05-2016 Agreed that the next step is further investigation, proposed method is to look at a range of samples with decreasing amounts of sperm in the presence of consistent amounts of epithelial cells and test: ER microscopy results, AP & p30 presumptive tests, Diff lysis slide microscopy results, quant results and profiling results.

- Look at current diff lysis pos control procedure and look at amounts that currently are added
- Look at previous p30 / AP validations for sensitivity of presumptive tests
- Modify pos control procedure to get swabs (mock samples) that contain decreasing amounts
  of semen; include a number of samples with excessive amounts of material; perform in a
  sufficient number of replicates to test reproducibility of ER method
- Pos controls are created using cell counts to determine dilution of semen to be added to swab, however relative amounts of spermatozoa to p30 and AP activity will be different between individuals, so maybe best to look at a range of dilutions of neat semen from a range of donors.
- Make swab suspensions as per current routine procedures
  - Do microscopy

- Irrespective of micro results, process through AP and p30 (departure from current procedure)
- o Process through Diff Lysis extraction
  - Stain and review each diff lysis slide
- Quant sperm frac only (extract & hold on efrac)
- Profile sperm frac only
- If sperm frac profiling result has carry over decon through STRmix and use modelled mixture proportion to calculate "sperm" quant value

#### Previous in house studies:

- Maxwell Diff Lysis extraction (project #85) Quotes that the limit of allele detection is 5,500 sperm cells (from two published sources) and showed reasonable quant values from 4 μL of a 1/100 semen dilution (approx. 5,500 sperm cells) and full 18 allele (Pro+) profiles at this level, and that a 100 μL of 1/20,000 semen dilution gave no profile
- ABA card p30 (project #95) showed positive results to a dilution of 1/1,000 to 1/50,000 of semen (nanopure water) and 1/50,000 when using the commercial extraction buffer.
   1/100,000 was negative for both methods, also showed that AP was detected to 1/100 when using nanopure water and to 1/1,000 when using the commercial buffer.
- Frozen AP (project #136) showed AP was detected to dilution of semen of 1/100 and was negative at 1/1,000
- RSID semen detection experiments showed p30 was detectable to 1/100 reliably and unreliably to 1/1,000.
- NO in-house data located which correlates sperm numbers added to swab against
  microscopy results, or correlates sperm microscopy results against profiling results, or
  correlates sperm dilutions (used for presumptive testing) with microscopy results.

#### Testing of a batch of 3 previously prepared positive extraction controls:

• Controls tested contained 8  $\mu$ L of a dilution of 1/20 of neat semen from donor

#### Possible mock swab creation:

- 20 μL of buccal cell suspension, 10 μL of the following dilutions of semen:
  - 0 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500
- Logic is that approx. 1/20 dilution should give a microscopy result of about 1+ according to
  pilot study of pos control samples, around 1/100 is approximately the limit of detection of

AP /p30 presumptive tests based on previous in-house studies, around 1/20 dilution yields a quant value of approximately 0.01 ng/ $\mu$ L (based on recent Diff Lysis pos control log), results in close to the 150ng of total DNA added to Amp which is at the stochastic threshold of the amplification kit. It would be expected therefore, that approximately 1/100 should give little to no DNA profile. However, Diff Lysis pos control batch results shows an average sperm lysate quant of 0.07 ng/ $\mu$ L ... ? carry-over effect – consistent difference between pos control log and batch results. Regardless, the indication is that a similar level of sensitivity appears to exist between micro, presumptive testing and STR profiling.

#### **Angelina Keller**

From: Matthew Hunt

Sent: Thursday, 27 September 2018 12:47 PM

To: Adrian Pippia; Allison Lloyd; Angelina Keller; Angela Adamson; Cassandra James; Jacqui Wilson;

Rhys Parry; Thomas Nurthen; Alicia Quartermain; Claire Gallagher; Deborah Nicoletti; Emma

Caunt; Hannah Pattison; Ingrid Moeller; Josie Entwistle; Penelope Taylor

Cc: Kylie Rika; Sharon Johnstone; Justin Howes; Paula Brisotto; Allan McNevin; Luke Ryan

**Subject:** Sperm fractions

Hi,

A few recent examples have been noted of samples from sexual assault cases (from HVS, bedding fabric, condom) which featured the following:

- 1: No sperm seen at ERT microscopy
- 2. Diff Slide positive for sperm (the examples had 2+ and 3+ heads)
- 3. Quant is 'DNA Insufficient for further Processing'

In each instance the reporter has opted to microcon these (sperm and/or epi) fractions and strong DNA profiles (Male SS or MIX) have resulted.

These examples have been brought to the attention of Paula and will be discussed with the Lab Team Managers.

Please note that the rework strategy undertaken for these samples may not be appropriate or effective in all cases, and as ever appropriate caution should be applied when interpreting profiles in the stochastic range, particularly from a single replicate.

Project #181 is still ongoing which is intended to improve the microscopy/sperm recovery process. In the meantime however, please keep an eye out for any similar examples.

Thanks,



#### **Matthew Hunt**

Acting Senior Scientist - Forensic Reporting and Intelligence Team

#### Forensic DNA Analysis, Forensic & Scientific Services

Health Support Queensland, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.





## Project #181 - History

- Concerns first raised by FRIT that ER slides may have lower sensitivity than Diff Lysis slides (2015)
- · Difference in sperm numbers at ERT microscopy vs Diff Lysis slide
- · Issue with sensitivity of ERT sampling and/or microscopy
- Difference in concentration at ER vs Diff Lysis slide a consideration? ~200uL at ER vs <50uL at D/L?</li>
- · Data comparison performed between initial microscopy and diff slide results:
  - · More sperm on diff slide (52)
  - More sperm on ERT slide (10)
  - · Concordant sperm on ERT/Diff slides (17)
- Risk mitigation put in place while project underway all samples with sperm negative undergo Differential Lysis extraction and Diff Lysis slide read for final sperm result.

\_

## Experiment Part 1 (June 2016)

- Apply decreasing amounts of semen to swabs (+ constant epis):
  - 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500 Dilutions
- Less sperm observed on ER slide than Diff slide
   Relatively small difference (not unexpected)
- Epis observed on ER slides: not being lost during slide prep
- · AP and p30 results as expected

2

# Part 2 – Proposed spin-basket processing

- Could sensitivity of ERT microscopy be improved?
- Are sperm being retained in the swab during ERT processing?
- Proposed new process. After ERT slide:
- Transfer swab to spin basket
- Centrifuge
- Transfer supernatant and spin basket swab to new tubes
- Resuspend pellet + make second slide
- Pellet & swab from spin basket for DNA profiling (separate samples)
- Results showed sperm were still being retained in swab during current and spin-basket processes

-

# Options – April 2018

### Mgmt team considered options:

- 1. Improve ERT process to release more sperm from swab
- 2. Submit swabs straight for Diff. Only proceed to DNA profiling if sperm detected at diff

## Agreed on modified version of Option 2:

- · Submit all swabs directly for diff
- Retain potential for presumptive testing
- Adapt ERT process to:
  - o Preserve sperm
  - o Allow AP, p30 and Phadebas testing

-

## Part 3 - Proposed Method

- Mock samples: replicates of 1/100, 1/200 and 1/500 neat semen, plus ~3 x epis, dried@35°C
- Half processed under current standard procedures (+ AP and p30 testing)
- · Half processed under proposed ERT process:
- Add 650µL nanopure water
- · Vortex, incubate (30 mins, RT)
- · Vortex, centrifuge 2 mins
- Transfer 150  $\mu$ L supernatant to new tube ("SUPNAT" for Phadebas)
- Transfer 300 µL to another tube ("MISC" for AP / p30)
   MISC & SUPNAT stored frozen.

### Part 3 - Results

- Proposed process gave comparable or improved sperm microscopy compared to ERT and diff slides
- Both Current and Proposed were AP positive at 1/100
- Current process was AP positive at 1/200
- Proposed process was AP negative at 1/200
- Both Current and Proposed were p30 positive at 1/100
- · Both Current and Proposed were Phadebas positive for all samples
- · Next steps to optimise Proposed process across a range of variables:
  - o Semen donors
  - o Dilutions
  - o Substrates
  - o Volume of water added in ERT
- o Incubation time/Temp variations



## Part 4 (i) Incubation Variables (2019)

· Compared Current process to Proposed under different incubation conditions:

15mins@RT; 30mins@RT; 15mins@30°C; 30mins@ 30°C

Mock swabs: replicates of 1/100; 1/200; 1/500 semen dilutions

#### Results Discussion:

- All Proposed processes comparable or better than Current (ERT) microscopy
- 30mins@RT and 15mins@30°C were optimum (microscopy comparable to diff slides and gave best p30 sensitivity)
- Current process AP+ve @ 1/100 (both replicates) but Proposed processes all AP-ve
- · All results Phadebas +ve
- Sperm microscopy was more sensitive than AP / p30 (across all results)

\*

## Part 4 – Further testing; AP

- · As 'Proposed processes' all AP-ve :
  - 1. Possible dilution effect on AP results?
  - 2. Does freezing supernatant affect AP?
- Used 5 x donors at 1/50, 1/100, 1/200, 1/500 semen dilutions
- Tested Current process and two 'best' Proposed processes (30 mins@RT and 15 mins@30°C)
- · Proposed processes tested as before; also tested AP before freezing of supernatant
- After completing this testing, a new batch of AP (with fresh sodium a-naphthyl phosphate) was prepared, and frozen samples were retested
- AP results remained unsatisfactory; no significant difference found between fresh or frozen samples, or when using freshly prepared AP

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# Part 4 - amended

- · Test whether reducing water affects AP and p30 sensitivity:
  - o Add 400uL of nano H20
  - o Remove 200uL for AP and p30
  - o Retain frozen and test after ~1week
  - o Submit all samples for DLYS retain supernatant (for phadebas)
- Further testing of different incubation conditions: Time; Temp and agitation (using thermomixer vs heatblock) for Current and Proposed processes
- Used Donor 5 for mock swabs: 1/20, 1/50, 1/100, 1/200, 1/500 dilutions (in duplicate)

10



## Results Discussion: Part 4 - amended

- Negligible impact to AP / p30 sensitivity by reducing water
- Microscopy results were very good for proposed process
- Best overall results (for both Micro + p30) were from 15 mins@30°C
- p30 results were in line with validation studies, once initial dilutions were considered
- AP results were still not as good for the proposed process (sample is ~2x as dilute as current process)

-

# Part 4 – recent testing

- Next we compared Current process to Proposed process at 15 mins@~30°C (best results) on different fabric types and using 5 x donors
- Kept reduced water volume (400uL) (efficiency saving as Phadebas required infrequently)
- Samples collected by scraping fabrics (initial stage option to tape-lift later)

#### Microscopy results

Microscopy	Ŧ	Swabs		Thick fabric	*	Thin fabric	¥	Total	*
Proposed process better than current process			1		5		1		7
Proposed process consistent with current proce	255						3		3
Proposed process worse than current process							1		1
Proposed process better than diff slide					3				3
Proposed process consistent with diff slide			1		1		1		3
Proposed process worse than diff slide					1		4		5

- The proposed process performed better than the current process.
- The results from the proposed process showed that the heat block was better than the thermomixer on 4 occasions, there was no difference between the two on 5 occasions and the thermomixer was better than the heat block on 1 occasion.

1.0



Thick fabric

Thin fabric

5

▼ Total

10

• Overall, the proposed process is consistent with the current process.

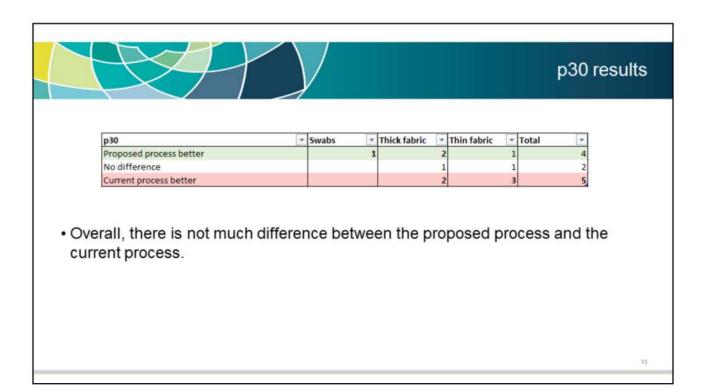
▼ Swabs

· Swabs:

Proposed process better No difference

Current process better

- o Current process detected AP to a sensitivity of 1/100, and was detectable at 1/200
- o Proposed process detected AP to a sensitivity of 1/50
- Note: The proposed process is twice as dilute as the current process. All results obtained were in line with validation studies once dilution factors were taken into account.
- No fabrics returned a positive AP result
- Proposed process: The thermomixer and heat block produced the same results





			Cu	rrent					Pro	posed		
	S	wabs	Thic	k fabric	Thin	fabric	5	iwabs	Thic	k fabric	Thir	fabric
	sensitivity	detection limit										
Donor 4			1/100	1/200	1/100	1/200			1/50	1/200	1/50	1/100
Donor 5	1/20	1/200	1/20	1/100	1/50	1/100	1/100	1/200	1/20	1/100	1/50	1/100
Volunteer 1			1/50	1/200	1/100	1/500			<1/20	1/500	1/200	
Volunteer 2			1/50		1/100	1/200			1/100		1/50	1/200
Volunteer 3			1/20	1/200	1/100	1/200			1/100	1/200	1/20	1/100

#### · Swabs:

- o Current Process: Sensitive to 1/20, detectable to 1/200
- o Proposed process: Sensitive to 1/100, detectable to 1/200

#### Fabrics

- o Variable level of sensitivity
- o Current Process: Best sensitivity 1/100, detectable to 1/500
- o Proposed Process: Best sensitivity 1/200, detectable to 1/500

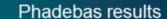
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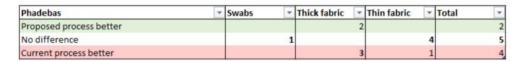


#### p30 results

p30	Swabs	Thick fabric	Thin fabric
Donor 4 - Thermomixer		2	2
Donor 4 - Heat Block		2	3
Donor 5 - Thermomixer	3	2	3
Donor 5 - Heat Block	2	2	2
Volunteer 1 - Thermomixer		3	4
Volunteer 1 - Heat Block		4	4
Volunteer 2 - Thermomixer		3	2
Volunteer 2 - Heat Block		3	3
Volunteer 3 - Thermomixer		3	1
Volunteer 3 - Heat Block		4	3

- The above graph is showing the positive results obtained from the thermomixer vs the heat block for the proposed process.
- The heat block was better on 5 occasions, their was no difference on 4 occasions, and the thermomixer was better on two occasions.





- The proposed process appears consistent with, or a little worse than the current process.
- Overall, the current process produced 18 positive results and the proposed process produced 15 positive results.
- Of those 15 positive results for the proposed process, the heat block produced 7 positive results while the thermomixer produced 8 positive results



#### Next Steps

- Tape-lifts of fabrics or further work on swabs (range of donors); excisions?
- Given fabric scraping results, not expecting tape-lifts to be as informative as swabs
- SAIK swabs cotton versus rayon
- Consider whether AP is still necessary (other than to locate stains on items)
- Reporters generally in favour of dropping AP if p30 can be relied on
- · Issue if p30 kit fails

# Cost – Dropping AP

- Data Analysis of previous 2 years: all FR exhibits until July 2019
- 1149 AP neg samples would need p30 (if AP dropped)
- ~\$9.32 per p30 test. 1149 x 9.32 = \$10,708.68
- 775 samples would no longer require AP (had both AP and p30)
- ~13c per AP test. 775 x 0.13 = \$100.75
- 10708.68 100.75 = \$10,607.93
- · Approx \$5,300 per annum



# Health Support Queensland

**Project # 181 Update** 

Matthew Hunt and Chelsea Savage 02/04/2020





# Project #181 – History

- Concerns first raised by FRIT that ER slides may have lower sensitivity than Diff Lysis slides (2015)
- Difference in sperm numbers at ERT microscopy vs Diff Lysis slide
- Data comparison performed between initial microscopy and diff slide results:
  - More sperm on diff slide (52)
  - More sperm on ERT slide (10)
  - Concordant sperm on ERT/Diff slides (17)
- Risk mitigation put in place while project underway all samples with sperm negative undergo Differential Lysis extraction and Diff Lysis slide read for final sperm result.



# Experiment Part 1 (June 2016)

- Apply decreasing amounts of semen to swabs (+ constant epis):
  - 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500 Dilutions
- Less sperm observed on ER slide than Diff slide
   Relatively small difference (not unexpected)
- Epis observed on ER slides: not being lost during slide prep
- AP and p30 results as expected



- Could sensitivity of ERT microscopy be improved?
- Are sperm being retained in the swab during ERT processing?
- Proposed new process. After ERT slide:
- Transfer swab to spin basket
- Centrifuge
- Transfer supernatant and spin basket swab to new tubes
- Resuspend pellet + make second slide
- Pellet & swab from spin basket for DNA profiling (separate samples)
- -Results showed sperm were still being retained in swab during current <u>and</u> spinbasket processes



# Options – April 2018

#### Mgmt team considered options:

- 1. Improve ERT process to release more sperm from swab
- 2. Submit swabs straight for Diff. Only proceed to DNA profiling if sperm detected at diff

#### Agreed on modified version of Option 2:

- Submit all swabs directly for diff
- Retain potential for presumptive testing
- Adapt ERT process to:
  - o Preserve sperm
  - o Allow AP, p30 and Phadebas testing



# Part 3 – Proposed Method

- Mock samples: replicates of 1/100, 1/200 and 1/500 neat semen, plus ~3 x epis, dried@35°C
- Half processed under current standard procedures (+ AP and p30 testing)
- Half processed under proposed ERT process:
- Add 650µL nanopure water
- Vortex, incubate (30 mins, RT)
- Vortex, centrifuge 2 mins
- Transfer 150 µL supernatant to new tube ("SUPNAT" for Phadebas)
- Transfer 300 μL to another tube ("MISC" for AP / p30)
   MISC & SUPNAT stored frozen.



### Part 3 - Results

- Proposed process gave comparable or improved sperm microscopy compared to ERT and diff slides
- Both Current and Proposed were AP positive at 1/100
- Current process was AP positive at 1/200
- Proposed process was AP negative at 1/200
- Both Current and Proposed were p30 positive at 1/100
- Both Current and Proposed were Phadebas positive for all samples
- Next steps to optimise Proposed process across a range of variables:
  - Semen donors
  - o Dilutions
  - Substrates
  - o Volume of water added in ERT
  - o Incubation time/ Temp variations



# Part 4 (i) Incubation Variables (2019)

- Compared Current process to Proposed under different incubation conditions:
- 15mins@RT; 30mins@RT; 15mins@30°C; 30mins@ 30°C
- Mock swabs: replicates of 1/100; 1/200; 1/500 semen dilutions

#### **Results Discussion:**

- All Proposed processes comparable or better than Current (ERT) microscopy
- 30mins@RT and 15mins@30°C were optimum (microscopy comparable to diff slides and gave best p30 sensitivity)
- Current process AP+ve @ 1/100 (both replicates) but Proposed processes all AP-ve
- All results Phadebas +ve
- Sperm microscopy was more sensitive than AP / p30 (across all results)



# Part 4 – Further testing; AP

- As 'Proposed processes' all AP-ve :
  - 1. Possible dilution effect on AP results?
  - 2. Does freezing supernatant affect AP?
- Used 5 x donors at 1/50, 1/100, 1/200, 1/500 semen dilutions
- Tested Current process and two 'best' Proposed processes (30 mins@RT and 15 mins@30°C)
- Proposed processes tested as before; also tested AP before freezing of supernatant
- After completing this testing, a new batch of AP (with fresh sodium a-naphthyl phosphate) was prepared, and frozen samples were retested
- AP results remained unsatisfactory; no significant difference found between fresh or frozen samples, or when using freshly prepared AP



### Part 4 - amended

- Test whether reducing water affects AP and p30 sensitivity:
  - o Add 400uL of nano H20
  - o Remove 200uL for AP and p30
  - Retain frozen and test after ~1week
  - Submit all samples for DLYS retain supernatant (for phadebas)
- Further testing of different incubation conditions: Time; Temp and agitation (using thermomixer vs heatblock) for Current and Proposed processes
- Used Donor 5 for mock swabs: 1/20, 1/50, 1/100, 1/200, 1/500 dilutions (in duplicate)



# Results Discussion: Part 4 - amended

- Negligible impact to AP / p30 sensitivity by reducing water
- Microscopy results were very good for proposed process
- Best overall results (for both Micro + p30) were from 15 mins@30°C
- p30 results were in line with validation studies, once initial dilutions were considered
- AP results were still not as good for the proposed process (sample is ~2x as dilute as current process)



# Part 4 – Scrapings

- Next we compared Current process to Proposed process at 15 mins@~30°C (best results) on different fabric types and using 5 x donors
- Kept reduced water volume (400uL) (efficiency saving as Phadebas required infrequently)
- Samples collected by scraping fabrics
- We presented these results to management and then did further testing on different sampling types (tape-lifts, excisions, cotton swabs) using 3 donors (donor 4, volunteer 1, volunteer 3).
- Combined results from this set of testing, as seen in the next few slides.



	Rayon		Scraping -	Scraping -	Tapelift -	Тар	elift -	Excision -	Excision -	Cotton		
Microscopy	Swabs	۳	Thick	Thin 💌	Thick -	Thi	n 💌	Thick 💌	Thin 💌	Swabs 💌	Total	w
Proposed process better than current ER slide		1		1		2	3	3	3	3	3	21
Proposed process consistent with current ER slide				3	3	1						4
Proposed process worse than current ER slide				1				1				1
Proposed process better than current diff slide			(3)					1	2	2	2	8
Proposed process consistent with current diff slide		1	1	. 1		L	2					6
Proposed process worse than current diff slide			1	. 4	1	2	1	2	1	1	L	12

- The proposed process performed better than the current ER slide
- Results were variable when comparing the proposed process against the current diff slide
- Overall, the heat block performed better than the thermomixer



	Rayon	Scraping -	Scraping -	Tapelift -	Tapelift -	Excision -	Excision -	Cotton	
AP	Swabs 💌	Thick	Thin 💌	Thick 💌	Thin 💌	Thick 💌	Thin 💌	Swabs 💌	Total 💌
Proposed process better						1			1
No difference		5	5	3	3			1	17
Current process better	1					2	3	2	8

- Overall the current process performed better
- None of the fabrics that were tape-lifted or scraped produced an AP positive result.
- Proposed process: The thermomixer and heat block produced comparable results (Thermomixer: 6 pos, Heat block: 5 pos)



	Current -	Rayon Swabs	Proposed -	- Rayon Swabs	Current -	Excision Thick	Proposed	- Excision Thick	Current	- Excision Thin	Proposed	- Excision Thin	Current	- Cotton Swabs	Proposed	- Cotton Swabs
	Sensitivity	Detection Limit	Sensitivity	Detection Limit	Sensitivity	Detection Limit	Sensitivity	Detection Limit	Sensitivity	Detection Limit						
Donor 4					1/20	1/20	1/20	1/50	1/20	1/20	AP neg	AP neg	AFneg	APneg	APneg	AP neg
Donor 5	1/100	1/200	1/50	1/50							- 2.		35			
Volunteer 1					1/50	1/50	1/20	1/20	Nil	1/20	AP neg	AP neg	Nil	1/20	APneg	AP neg
Volunteer 3					1/50	1/50	1/20	1/20	Nil	1/50	AP neg	AP neg	Nil	1/20	APneg	AP neg

# 4 (out of 8) substrates / sampling techniques produced positive results Rayon Swabs:

- ★ Current process: Sensitivity 1/100, detection limit 1/200
- Proposed process: Sensitivity 1/50, detection limit 1/50

#### **Excisions of Thick Fabric:**

- ★ Current process: Best sensitivity 1/50, best detection limit 1/50
- Proposed process: Best sensitivity 1/20, best detection limit 1/50

#### **Excision of Thin Fabric:**

- ★ Current process: Best sensitivity 1/20, best detection limit 1/50
- Proposed process: All AP negative

#### Cotton Swabs:

- \* Current process: Nil sensitivity, best detection limit 1/20
- Proposed process: All AP negative



	Rayon	Scraping -	Scraping -	Tapelift -	Tapelift -	Excision -	Excision -	Cotton	
p30	Swabs 💌	Thick	Thin 💌	Thick 💌	Thin 💌	Thick 💌	Thin 💌	Swabs 💌	Total 💌
Proposed process better	1	2	1				3	2	9
No difference		1	1	3	3	3			11
Current process better		2	3					1	6

- Overall the proposed processed performed better
- None of the fabrics that were tape-lifted produced a p30 positive result
- Proposed process: The heat block was a little better than the thermomixer (Heat block: 71, thermomixer: 65).



	Current -	Rayon Swabs	Proposed	- Rayon Swabs	Current -	Scraping Thick	Proposed	- Scraping Thick	Current -	- Scraping Thin	Proposed	· Scraping Thick
	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit						
Donor 4					1/100	1/200	1/50	1/200	1/100	1/200	1/50	1/100
Donor 5	1/20	1/200	1/100	1/200	1/20	1/100	1/20	1/100	1/50	1/100	1/50	1/100
Volunteer 1					1/50	1/200	<1/20	1/500	1/100	1/500	1/200	1/200
Volunteer 2					1/50	1/50	1/100	1/100	1/100	1/200	1/50	1/200
Volunteer 3					1/20	1/200	1/100	1/200	1/100	1/200	1/20	1/100
	Current - (	Cotton Swabs	Proposed	- CottonSwabs	Current -	Excision Thick	Proposed	l - Excision Thick	Current	- Excision Thin	Proposed	- Excision Thin
	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit						
Donor 4	1/20	1/100	1/100	1/200	1/500	1/500	1/500	1/500	1/200	1/200	1/500	1/500
Volunteer 1	1/20	1/50	1/50	1/50	1/500	1/500	1/500	1/500	1/200	1/200	1/500	1/500
Volunteer 3	1/50	1/200	1/50	1/50	1/500	1/500	1/500	1/500	1/100	1/100	1/200	1/500

• 6 (out of 8) substrates / sampling techniques produced positive results

#### Rayon Swabs:

- Current process: Sensitivity 1/20, detection limit 1/200
- Proposed process: Sensitivity 1/200, detection limit 1/200 Scraping of Thick Fabric
- Current process: Best sensitivity 1/100, best detection limit 1/200
- Proposed process: Best sensitivity 1/100, best detection limit 1/500 Scraping of Thin Fabric
- Current process: Best sensitivity 1/100, best detection limit 1/500
- Proposed process: Best sensitivity 1/200, best detection limit 1/200

#### Cotton Swabs:

- Current process: Best sensitivity 1/50, best detection limit 1/200
- Proposed process: Best sensitivity 1/100, best detection limit 1/200 Excision of Thick Fabric
- Current process: Best sensitivity 1/500, best detection limit 1/500
- Proposed process: Best sensitivity 1/500, best detection limit 1/500 Excision of Thin Fabric
- Current process: Best sensitivity 1/200, best detection limit 1/200
- Proposed process: Best sensitivity 1/500, best detection limit 1/500



### Phadebas results

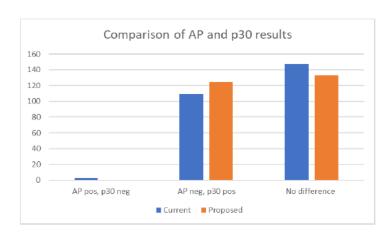
	Rayon	Scraping -	Scraping -	Tapelift -	Tapelift -	Excision -	Excision -	Cotton	
Phadebas	Swabs 🖪	Thick 💌	Thin 💌	Thick 💌	Thin 💌	Thick 💌	Thin 💌	Swabs 💌	Total 💌
Proposed process better		2							2
No difference		L	4	3	3	2	2	2	17
Current process better		3	1			1	1	1	7

- The proposed process appears consistent with, or a little worse than the current process. However, overall the current process produced <u>118</u> positive results compared to <u>111</u> for the proposed process (260 samples were tested for each process) - Not a huge difference
- No fabrics that were tape-lifted produced a phadebas positive result
- Proposed process: The thermomixer and heat block produced comparable results (thermomixer: 56 pos, heat block: 55 pos).



# Next Steps

- Consider whether AP testing on subsamples is worthwhile moving forward.
  - o P30 testing outperformed AP testing
    - Current process: AP pos, p30 neg: 3 samples
    - Current process: AP neg, p30 pos: 109 samples
    - Proposed process: AP pos, p30 neg: 0 samples
    - Proposed process: AP neg, p30 pos: 125 samples
  - o AP testing will still be retained for whole item screening
  - o Reporters generally in favour of dropping AP if p30 can be relied on
  - o Cost implications





- Should we quant the results that we have?
  - o There is a risk that we are losing DNA on the slide. We could therefore quant our data and look at the Y quant vs the total quant to ensure male DNA is still present in the sample, and not all of it is ending up on the slide.
  - We could either quant only those with a lower sperm count (0, <1+, 1+) as there is potentially a higher risk of losing DNA when there is less sperm present, or we could quant everything. (481 samples vs 520 samples).
- Do you consider sufficient work has been performed to finish the project here?

Mock sample creation

Semen control serial dilutions			volume to add t	o swab	total vol req'd	d (4 swabs of each)
final conc	vol of semen	vol n. H20	semen dilution	epi cells	semen dilutio	n epi cells
1 in 5	10uL neat semen	40uL	5uL	50uL	20uL	200uL
1 in 10	25uL 1 in 5 dil'n	25uL	5uL	50uL	20uL	200uL
1 in 20	25ul 1 in 10 dil'n	25uL	5uL	50uL	20uL	200uL
1 in 50	20uL 1in 20 dil'n	30uL	5uL	50uL	20uL	200uL
1 in 100	25uL 1 in 50 dil'n	25uL	5uL	50uL	20uL	200uL
1 in 200	25uL 1 in 100 dil'n	25uL	5uL	50uL	20uL	200uL
1 in 500	20uL 1in 200 dil'n	30uL	5uL	50uL	20uL	200uL
					10uL semen c	o 1.4mL buccal control

Semen control details:

Cell control details:

FBOT+ to get UR no (same UR for all samples)
Surname = Project 181
Sample type = SFRAC & EFRAC
check with LBR / PA - ?need to use VDFLYS - Validation Diff Lysis batch type
sample info = dilution details
Processing comment = ext & hold all (sfracs and efracs)

Intial testing:

						ER Slide			Diff Slide	
Barcode	Dilution	AP	AP time (sec)	p30	whole sperm	sperm heads	epithelials	whole sperm	sperm heads	epithelials
	1 in 5 #1	pos	40	neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 5 #2	pos	20	pos	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 5 #3	pos	45	pos	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 5 #4	pos	45	pos	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 10 #1	pos	35	neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 10 #2	pos	40	neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 10 #3	pos	40	neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 10 #4	neg		pos	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 20 #1	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 20 #2	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 20 #3	pos	110	neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 20 #4	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 50 #1	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 50#2	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 50 #3	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 50 #4	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 100 #1	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 100 #2	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 100 #3	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 100 #4	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 200 #1	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 200 #2	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 200 #3	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 200 #4	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 500 #1	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 500 #2	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 500 #3	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A
	1 in 500 #4	neg		neg	N/A	N/A	N/A	N/A	N/A	N/A

Note: The negative results recorded in this table were flagged as unexpected at the time of testing as they are inconsistent with our P30 validation. A decision was made at the time to read the slides to give us further insight into the samples. This was due to be done on 07/02/2017.

A decision was made by JAH/EJC/AR to halt testing on 07/02/2017. Further discussion suggested a possible degraded semen sample, or the samples were too dilute. RJP re-calculated the dilution factor and the 1/5 semen dilution was actually 1/250 with the addition of 50uL of epi cells. This would explain the negative AP and P30 tests with more dilute samples.

Further discussions are required and a new experimental design will be proposed. AR 08/02/2017

						ER Slide			Diff Slide		
arcode	Dilution	AP	AP time (sec)	p30	whole sperm	sperm heads	epithelials	whole sperm	sperm heads	epithelials	
	1 in 5 #1	pos	25	pos	2	2	1	<1	3	0	
	1 in 5 #2	pos	20	pos	1	2	3	0	3	0	
	1 in 5 #3	pos	20	pos	1	2	3	0	3	0	
	1 in 5 #4	pos	20	pos	1	1	3	<1	3	0	
	1 in 10 #1	pos	35	pos	<1	<1	2	0	3	0	
	1 in 10 #2	pos	30	pos	<1	1	3	0	3	0	
	1 in 10 #3	pos	30	pos	<1	1	1	0	3	0	
	1 in 10 #4	pos	35	pos	1	2	3	0	3	0	
	1 in 20 #1	pos	30	NEG	1	1	3	0	2	0	
	1 in 20 #2	pos	50	pos	1	1	3	0	2	0	
	1 in 20 #3	pos	45	pos	1	1	3	0	2	0	
	1 in 20 #4	pos	60	pos	0	<1	2	0	2	0	
	1 in 50 #1	pos	60	pos	<1	<1	3	0	1	0	
	1 in 50 #2	pos	110	pos	<1	<1	3	0	1	0	
	1 in 50 #3	pos	100	NEG	0	<1	3	0	1	0	
	1 in 50 #4	pos	70	NEG	0	<1	3	0	1	0	
	1 in 100 #1	pos	110	NEG	0	<1	3	0	1	0	
	1 in 100 #2	NEG		NEG	0	<1	2	0	1	0	
	1 in 100 #3	pos	120	NEG	0	0	1	0	1	0	
	1 in 100 #4	pos	110	pos	<1	<1	3	0	1	0	
	1 in 200 #1	NEG		NEG	0	0	2	0	<1	0	
	1 in 200 #2	NEG		NEG	0	0	3	0	1	0	
	1 in 200 #3	NEG		NEG	0	<1	3	0	1	0	
	1 in 200 #4	NEG		NEG	0	<1	1	0	1	0	
	1 in 500 #1	NEG		NEG	0	<1	3	0	<1	0	
	1 in 500 #2	NEG		NEG	0	<1	3	0	<1	0	
	1 in 500 #3	NEG		NEG	0	<1	3	0	<1	0	
	1 in 500 #4	NEG		NEG	0	0	3	0	<1	0	

Samples:

(24 samples)

1 in 100 to 1 in 700 / 750

increase replicates to 6 for each dilution

Use spin basket spin settings as per Analytical procedures

No need to further test AP /p30

Proposed next step - to be discussed next mngmt meeting  $^{\sim}16/3$ 

- 1. Create new set of samples
- 2. Sample swab head into a flip-top tube
- 3. Create suspension into flip-top tube (mirrors current procedure but in a different tube)
- 4. make a micros slide as per current procedure
- 5. transfer swab material to a spin basket, & spin
- 6. put spin basket contents and most of supernatant into individual new screw-top tubes
- 7. re-suspend pellet & make a new slide
- 8. submit pellet to Analytical in flip-top tube for diff lysis
- 9. Look at Diff Lysis slide
- 10. Submit ER spin basket (without supernatant) for diff lysis
- 11. Look at Diff Lysis

#### Questions to answer

- Q1. Do you get a better slide from ER than current process?
- Q2. Do you lose sperm from the diff process by seeing more at ER? (could that be mitigated by stopping diff slides in the future)
- Q3. Is there sperm being retained in the ER spin basket?
- Q4. Does more ER "processing" cause a loss of tails?

#### Considerations

- 1. If new process works are the tube types a problem
- 2. If new process doesn't work (i.e. too much sperm lost to ER method or too much sperm retained in swab), will need to consider alternatives
- 2a. Some sort of Y-screening (Y-quant or Y-STR) on suspensions
- may be time consuming (P1 SAIKs)
- Y DNA may not be from Sperm
- 2b. Look at further tweaking the ER process
- Spin times
- Incubation times / temperatures (does that then affect AP /p30?)
- may be as simple as using more liquid before spin basket step but this would further dilute AP /  $p30\,$
- 3. Any further validation of a method would at least need to include:

fabric excision fabric scrape

tape-lift

ARM & EJC agreed - A new lot of samples to made using a fresh collection of semen & buccal cells and when making the swabs, the epi cells will be added first & dried at 35oC (rather than 50oC) then semen added and dried at 35oC ARM 17/02/2017

Swab creation, sampling, AP, p30 testing and making ER slides performed by CKS
ER slide reading and Diff Slide reading performed by AR
Diff Lysis batch processing performed by AK

#### Mock samples for experiment 2 recorded in the FR as case FR1714574

_	Exhibit Description	description	
ı	1:50 dilution (1)	[A] (SLIDE)	
۱	1:50 dilution (2)	[A] (SLIDE)	
ı	1:50 dilution (3)	[A] (SLIDE)	Mod
ı	1:50 dilution (4)	[A] (SUDE)	
ı	1:50 dilution (5)	[A] (SLIDE)	
ı	1:50 dilution (6)	[A] (SLIDE)	
ı	1:100 dilution (1)	[A] (SUDE)	
ı	1:100 dilution (2)	[A] (SLIDE)	
ı	1:100 dilution (3)	[A] (SLIDE)	
ı	1:100 dilution (4)	[A] (SUDE)	
ı	1:100 dilution (5)	[A] (SUDE)	
ı	1:100 dilution (6)	[A] (SUDE)	
١	Swab in spin basket [1:50 dilution (1)]	N/A	
ĺ	Swab in basket [1:50 dilution (2)]	N/A	
ı	Swab in basket [1:50 dilution (3)]	N/A	
ı	Swab in basket [1:50 dilution (4)]	N/A	
١	Swab in basket [1:50 dilution (5)]	N/A	
ı	Swab in basket [1:50 dilution (6)]	N/A	
l			
ı			
l	Swab in basket [1:100 dilution (1)]	N/A	
l	Swab in basket [1:100 dilution (2)]	N/A	
l			
ı	Swab in basket [1:100 dilution (3)]	N/A	
١	and in body for one display (all)		
١	Swab in basket [1:100 dilution (4)]	N/A	
۱	Swab in basket [1:100 dilution (5)]	N/A	
ı	Swab in basket [1:100 dilution (6)]	N/A	
ı	Resuspended pellet post spin [1:50 dilution(1)]	[A] (SLIDE)	
۱	Resuspended pellet post spin [1:50 dilution(2)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:50 dilution(3)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:50 dilution(4)]	[A] (SLIDE)	
۱	Resuspended pellet post spin [1:50 dilution(5)]	[A] (SLIDE)	
۱	Resuspended pellet post spin [1:50 dilution(6)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:100 dilution(1)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:100 dilution(2)]	[A] (SLIDE)	
۱	Resuspended pellet post spin [1:100 dilution(3)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:100 dilution(4)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:100 dilution(5)]	[A] (SLIDE)	
ı	Resuspended pellet post spin [1:100 dilution(6)]	[A] (SLIDE)	

iption																		
LIDE)																		
LIDE)	Swa	ab / Sample	ids				ER Slide					rom pellet)		ide (swab in spin	basket)		Diff Slide (pellet)	
LIDE)	Mock swab	Dilution	Swab spin bskt	pellet	Slide Barcode	whole sperm	sperm head	epithelials	Slide Barcode	whole sperm	sperm head	epithelials	Slide Barcode	sperm heads	epithelials	Slide Barcode	sperm heads	epithelials
LIDE)		1 in 50 #1	727542414	72754253		2	3	4		1	2	4		2	0		1	0
LIDE)		1 in 50#2	727542420	72754254		1	2	4		1	1	3		2	0		1	0
LIDE)		1 in 50#3	727542431	72754255		2	2	3		<1	1	3		2	0		1	0
LIDE)		1 in 50 #4	727542442	72754256		1	2	4		<1	1	4		1	0		1	0
LIDE)		1 in 50 #5	727542458	72754257		2	3	4		<1	1	2		1	0		2	0
LIDE)		1 in 50#6	727542469	72754258		1	3	4		<1	2	4		2	0		2	0
LIDE)		1 in 100 #1	727542475	72754259		1	2	4		<1	1	4		1	0		1	0
LIDE)		1 in 100 #2	727542486	72754260		1	1	4		<1	1	4		1	0		1	0
LIDE)		1 in 100 #3	727542497	72754261		<1	1	3		<1	1	4		1	0		1	0
		1 in 100 #4	727542506	72754262		1	1	4		<1	1	3		1	0		1	0
		1 in 100 #5	727542515	72754263		1	1	4		<1	1	3		1	0		1	0
		1 in 100 #6	727542529	72754264		1	1	4		<1	1	3		1	0		1	0
																	1	

b / Sa	mple ids		ER Slide		ER Slide po	ost spin (i.e. f	rom pellet)	Diff Slide (s	swab in spin	Diff Slid	le (pellet)
			sperm		whole	sperm		sperm		sperm	
	Dilution	whole sperm	heads	epithelials	sperm	heads	epithelials	heads	epithelials	heads	epithelials
	1 in 50 #1	2	3	4	1	2	4	2	0	1	0
	1 in 50 #2	1	2	4	1	1	3	2	0	1	0
	1 in 50 #3	2	2	3	<1	1	3	2	0	1	0
	1 in 50 #4	1	2	4	<1	1	4	1	0	1	0
	1 in 50 #5	2	3	4	<1	1	2	1	0	2	0
	1 in 50#6	1	3	4	<1	2	4	2	0	2	0
	1 in 100 #1	1	2	4	<1	1	4	1	0	1	0
	1 in 100 #2	1	1	4	<1	1	4	1	0	1	0

Using a spin basket to create a pellet at initial analysis does not appear to be adventageous
 Significant numbers of sperm are retained in the swab head after initial ER processing

4. In this experiment, no instance of diff slide showing higher numbers than ER processing slide, ? Due to double up of pre-processing

3. Point 1. is probably due to the effect of point 2. ??

Discussion 10/04

Sperm stuck on swab after initial suspension made

Option A - improve suspension making method to try to release more sperm

Option B - straight to diff, only proceed to profiling if sperm present

see over to the right for pros and cons

Swabs created from a fresh collection from the semen and buccal cells donors. Swabs created, processed and slides all read by NLR

Option A		Option B	
Pro	Con	Pro	Con
More efficient downstream from		Not losing sperm during screening	
ER	May slow down ER	= maximise profile recovery	losing capability to do AP / p30
May eliminate need to make diff	Further method development		Doesn't help analytical with diff
slide	req'd	Could implement straight away	extraction numbers
maintain AP/p30 capacity		speeds up initial processing in ER / reduces microscopy Could use Y-quant to assist in	Requires some FR / workflow development
cheaper		workflow choice	

#### Mock samples for experiment 3 recorded in the FR as case FR1749437

Eshibit Description
(1) 1/100 dilution - current process
(2) 1/100 dilution - current process
(3) 1/100 dilution - experimental process
(4) 1/100 dilution - experimental process
(3) 1/200 dilution - current process
(3) 1/200 dilution - current process
(3) 1/200 dilution - current process
(4) 1/200 dilution - current process
(3) 1/500 dilution - current process
(3) 1/500 dilution - experimental process
(3) 1/500 dilution - experimental process
(4) 1/500 dilution - experimental process
(4) 1/500 dilution - experimental process

mple ID	Semen dil'n	Slide barcode	whole	spi sperm	he epithelials	AP	time (s)	p30	Phadebas	Diff Slide barcode	Sperm	Sample ID	Semen dil'n	Diff Slide barcode	Sperm	AP	time (s) p30	Phadebas
	L/100		+	<1+	3+	pos	58	neg	pos		1+		/100		2+	pos	60 neg	pos
	1/100		1+	<1+	3+	pos	60	neg	pos		1+		/100		2+	pos	65 pos	pos
	L/200		1+		0 2+	pos	87	neg	pos		<1+		/200		1+	neg	neg	pos
	L/200		1+	<1+	3+	pos	104	neg	pos		<1+		/200		2+	neg	neg	pos
	L/500		1+		0 3+	neg		neg	pos		<1+		/500		1+	neg	neg	pos
	L/500		1+	<1+	2+	neg		neg	pos		<1+		/500		1+	neg	neg	pos

				Current p	rocess							Pro	posed pro	AP time (s) p30 Phadebas   pos 60 neg pos pos 65 pos pos neg N/A neg N/A neg neg N/A			
	Semen	whole	sperm						Sperm		Semen						
ample ID	dilution	sperm	heads	epithelials	AP	time (s)	p30	Phadebas	(diff slide)	Sample ID	dilution	Sperm	AP	time (s)	p30	Phadeba:	
	1/100	1+	<1+	3+	pos	58	neg	pos	1+		1/100	2+	pos	60	neg	pos	
	1/100	<1+	<1+	3+	pos	60	neg	pos	1+		1/100	2+	pos	65	pos	pos	
	1/200	<1+	0	2+	pos	87	neg	pos	<1+		1/200	1+	neg	N/A	neg	pos	
	1/200	<1+	<1+	3+	pos	104	neg	pos	<1+		1/200	2+	neg	N/A	neg	pos	
	1/500	<1+	0	3+	neg	N/A	neg	pos	<1+		1/500	1+	neg	N/A	neg	pos	
	1/500	<1+	<1+	2+	neg	N/A	neg	pos	<1+		1/500	1+	neg	N/A	neg	pos	
					_										_		

Dort	4-	Current	proces

Semen dil'n	Slide barcode	whole sperm	sperm heads	epithelials	AP	time (s)	p30	Phadebas	Diff Slide barcode	Sperm
1/100		0	<1+	1+	pos	1 min	neg	pos		2+
1/100		<1+	1+	2+	pos	1 min	pos	pos		1+
1/200		0	<1+	1+	neg		neg	pos		1+
1/200		0	<1+	1+	neg		neg	pos		1+
1/500		<1+	0	1+	neg		neg	pos		<1+
1/500		0	<1+	1+	neg		neg	pos		<1+

Part 1: 15 mins @ RT					
Semen dil'n piff Slide barcode	Sperm	AP	time (s)	p30	Phadebas
1/100	1+	neg		pos	pos
1/100	1+	neg		pos	pos
1/200	<1+	neg		neg	pos
1/200	<1+	neg		neg	pos
1/500	0	neg		neg	pos
1/500	<1+	neg		neg	pos
part 1: 30 mins @ RT					
Semen dil'n piff Slide barcode	Sperm	AP	time (s)	p30	Phadebas
1/100	1+	neg		pos	pos
1/100	2+	neg		pos	pos
1/200	1+	neg		pos	pos
1/200	<1+	neg		pos	pos
1/500	<1+	neg		neg	pos
1/500	<1+	neg		neg	pos
part 1: 15 mins @ ~30 degrees of	elsius				
Semen dil'n piff Slide barcode	Sperm	AP	time (s)	p30	Phadebas
1/100	2+	neg		pos	pos
1/100	2+	neg		neg	pos
1/200	1+	neg		pos	pos
1/200	<1+	neg		pos	pos
1/500	<1+	neg		neg	pos
1/500	<1+	neg		neg	pos

Observations
30min at RT and 15 min at 30 appear best, lost AP pos at 1/100, but gained p30 pos at 1/200
Phadebas results OK for everything
1/500 15min at RT worst, all other same, maybe slightly better for 30min at 30deg
Experiment 3, had AP pos at 1/100 for previous donor with new method and 1/200 with current process
Sperm microscopy more sensitive than AP / p30 across all experiments

Next step

- use all donors (this one, old previous, and 3 volunteers) to create 1/50, 1/100, 1/200, 1/500 swabs as three lots of duplicates

- sample and create slides, and do AP test on one set of duplicates as per current method

- sample and take off supernatants as per proposed method for 30min at RT and 15min at 30deg, perform AP test straight away on one part of supernatants, freeze remaining and retest frozen supernatant after same time period as before and retain frozen the sampled swab and supernatant for potential further testing if required

- agreed that testing of the Phadebas further does not appear useful hypothesis:

HI: there is a dilution effect on the AP results in this and part 3 of project experiments

H2: the freezing and testing AP affecting the AP results

Experiment 4 - part 1 stage 2: Current process    Semen dil'n	Sample ID   Semen dii'n   AP   time(s)	Volunteer 1   Semen dii'n   AP   time(s)   1/50   neg   1/50   pos nin 40 secs   1/100   neg   1/1	Volunteer 2   Sample ID   Semen dil'n   AP   time(s)	Volunteer 3  Sample ID  Semen dif'n  1/50  pos pini 20 secs  1/500  neg  1/100  neg  1/100  neg  1/200  neg  1/200  neg  1/500  neg
Semen dil'n   Initial AP   time(s)   AP after 1 week   time(s)	Semen dii'n	Semen dil'n	Semen dil'n	Semen dil'n
Semen dil'n   Initial AP   time(s)   AP after 1 week   time(s)	Semen dii'n	Semen dil'n	Semen dil'n	Semen dil'n
- part 1 stage 2: 30 mins @ RT - new AP reagent [14 days]  Semen dii'n New AP reagent time(s)  1/50 neg  1/50 neg  1/100 neg  1/200 neg  1/200 neg  1/300 neg  1/300 neg  1/300 neg  1/300 neg  1/500 neg  1/500 neg	Semen dil'n New AP reagent time(s)  1/50 neg  1/50 neg  1/100 neg  1/100 neg  1/100 neg  1/200 neg  1/200 neg  1/500 neg  1/500 neg  1/500 neg	Semen dil'n   New AP reagent   time(s)   1/50   neg   1/50   neg   1/100   neg   1/100   neg   1/200   neg   1/200   neg   1/500   neg   1/5	Semen dil   New AP reagent   time(s)   1/50   neg   1/50   neg   1/100   neg   1/100	Semen dii'n   New AP reagent   time[s]     1/50   neg     1/50   neg     1/100   neg     1/100   neg     1/200   neg     1/200   neg     1/500   neg     1/500   neg     1/500   neg
- part 1 stage 2: 15 mins @ 30 degrees celsius - new AP reagent {14 days}  Semen dil'n   New AP reagent   time(s)  1/50   neg  1/50   neg  1/100   neg  1/200   neg  1/200   neg  1/200   neg  1/500   neg  1/500   neg  1/500   neg  1/500   neg	Semen din   New AP reagent   time(s)   1/50   neg   1/50   pos   2 mins   1/100   neg   1/100   neg   1/100   neg   1/200   neg   1/200   neg   1/500   ne	Semen dil'n New AP reagent time(s) 1/50 neg 1/50 neg 1/100 neg 1/100 neg 1/200 neg 1/200 neg 1/200 neg 1/500 neg 1/500 neg	Semen dii'n   New AP reagent   time(s)   1/50   neg   1/50   neg   1/300   neg   1/300   neg   1/200   neg   1/2	Semen dil'n New AP reagent time(s) 1/50 neg 1/50 neg 1/100 neg 1/100 neg 1/100 neg 1/200 neg 1/200 neg 1/500 neg 1/500 neg

Semen dil'n	t process Slide barcode	whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	г
1/20		whole sperm	sperm neads <1+	epitheliais <1+		20 secs		DITI SIIGE DATCOGE	⊢
1/20		0	<1+	<1+	pos	30 secs	pos pos		⊢
1/50		0	<1+	0	pos	1 min	neg		H
1/50		0	<1+	1+	pos	1 min	pos		Г
1/100		0	<1+	1+	pos	1 min 10 secs	neg		Г
1/100		<1+	<1+	0	pos	1 min 10 secs	neg		Г
1/200		<1+	<1+	1+	pos	1 min 30 secs	pos		L
1/200		<1+	1+	1+	neg		neg		L
1/500		0	0	<1+	neg		neg		⊢
1/500	727897114	0	0	1+	neg		neg		L
Amended: Curren	t process with incubat	tion for 30mins	@ RT						
Semen dil'n			sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Г
1/20		<1+	<1+	1+	pos	30 secs	pos		Г
1/20		0	<1+	<1+	pos	25 secs	pos		Г
1/50		0	<1+	<1+	pos	1 min	neg		L
1/50		<1+	1+	1+	pos	1 min	pos		L
1/100		0	<1+	<1+	pos	1 min 30 secs	neg		⊢
1/100 1/200		<1+	<1+	1+ <1+	neg neg		neg neg	721044027	⊢
1/200	1	<1+	<1+	<li>41+</li>	neg		neg	721011027	H
1/500		0	<1+	<1+	neg		neg		H
1/500		0	0	<1+	neg		neg		Г
									Т
Amended: Curren		ion for 30 mins							_
Semen dil'n		whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	L
1/20		<1+	1+	1+	pos	30 secs	pos		⊢
1/20		<1+ 0	1+ 0	1+ <1+	pos	50 secs 1 min	pos neg		$\vdash$
1/50		0	0	- 1+	pos	1 min 50 secs	neg		H
1/100		0	<1+	<1+	pos	1 min 35 secs	neg		H
1/100		0	<1+	0	pos	2 mins	neg		r
1/200		0	<1+	0	pos	2 mins	neg		Г
1/200		0	<1+	1+	neg		neg		L
1/500		0	0	0	neg		neg		L
1/500	1	0	0	0	neg		neg		L
Amended: Propos		RT						Proposal for Expe	nim
Semen dil'n		Sperm	AP	time (s)	p30	Phadebas	l	rioposarioi Expe	
1/20		3+	pos	50 secs	pos	pos		Test whether redu	cin
1/20		4+	pos	50 secs	pos	pos		Amend proposed p	oro
1/50		1+	pos	2 mins	pos	pos		Remove 200uL for	Αp
1/50		2+	pos	1 min 30 secs	pos	pos		retain frozen and t	
1/100		2+	neg		pos	pos		Submit all samples	fo
1/100 1/200		1+ 2+	neg		neg	pos		Europhan taction of	
1/200		1+	neg neg		pos neg	pos		Further testing of Samples to be agit	
1/500		<1+	neg		neg	pos	'	oumpies to be ugit	300
1/500		1+	neg		neg	pos		Use Donor 5 to to	cre
Amended: Propos	-	RT							
Semen dil'n		Sperm	AP	time (s)	p30	Phadebas			
1/20		3+ 3+	pos	1 min 10 secs	pos	pos			
1/20 1/50		2+	pos	1 min 10 secs 1 min 30 secs	pos	pos			
1/50		1+	pos	1 min 50 secs	pos	pos			
1/100		1+	neg		neg	pos			
1/100		1+	neg		pos	pos			
1/200		1+	neg		neg	pos			
1/200		1+	neg		neg	neg			
1/500		<1+	neg		neg	pos			
1/500		<1+	neg		neg	neg	l		
- Amended: Propos	ie.	~30 degrees o	elsius					17024-2019042	4
Semen dil'n	-	Sperm	AP	time (s)	p30	Phadebas	l	17494-2328070	_
1/20		3+	pos	1 min	pos	pos		27 13 1 23200/0	_
1/20		4+	pos	1 min	pos	pos			
1/50		2+	pos	1 min 50 secs	pos	pos			
1/50		3+	pos	1 min 40 secs	pos	pos			
1/100		1+	neg		pos	pos		Discussion:	
1/100 1/200		1+	neg		pos	pos		Micro results are v	
1/200	•	1+	neg neg		neg pos	pos		After performing s It was also discuss	
1/500		<1+	neg neg		neg	pos		Moving forward: 0	
1/500		<1+	neg		neg	pos		Use 5 x donors	
,						, , ,	•	Scrape fabrics first	
- Amended: Propos		~30 degrees o						It was discussed th	at
Semen dil'r	+	Sperm	AP	time (s)	p30	Phadebas		Also discussed not	•
1/20		3+	pos	55 secs	pos	pos		1/20 dilution will n	ot
1/20	2	4+	pos	1 min 10 secs	pos	neg	,	Heden 24 (07/00)	
1/50		2+	pos	1 min 25 secs	pos	pos		Update 31/07/201	9::
1/50 1/100		1+ 1+	neg neg		pos pos	pos			
1/100			neg				l		
1/100	<u> </u>	1+	neg		neg	neg			
1/100 1/200		1+ 1+	neg neg		neg neg	neg neg			
1/100 1/200 1/200		1+ 1+ 1+	neg neg neg		neg neg neg	neg neg neg			

Proposal for Experiment 4 - Amended:

Test whether reducing water added to swab improves AP and P30 sensitivity Amend proposed process by adding 400uL of nano H20 (instead of 650uL) to swab. Remove 200uL for Ap and P30 testing

Phadebas

pos

pos pos pos pos

pos

pos pos pos pos

Phadebas

pos pos pos pos pos

retain frozen and test ~1week

Submit all samples for DLYS retain supernatant (for phadebas)

Sperm

Further testing of variation in incubation conditions (time; temperature; agitation) to see if this affects AP and P30 sensitivity Samples to be agitated in heated shakers

Use Donor 5 to to create 1/20, 1/50, 1/100, 1/200, 1/500 swabs as duplicates

17024-20190424\_MAM 17494-23280702

Micro results are very good for proposed process. Best overall results (micro + P30) at 15 mins @ ~30 degrees celsius

After performing some calculations, we were able to determine that the P30 results obtained were in line with validation studies once initial dilutions were considered

It was also discussed that the AP results are not as good in the proposed process, as they are performed using a sample which is twice as dilute as the current process Moving forward: Compare the current process to the proposed process - 15 mins @ ~30 degrees celsius (this has the best results) on different fabric types

Scrape fabrics first, if these results are good then move on to tape-lifting fabric

It was discussed that 5 out of 6 of the negative phadebas results came from samples incubated on a thermomixer. Samples to be split between the thermomixer and heat-block again for next round of testing. Thermomixer results seemed to be better than heat-block results for P30 and micro Also discussed not performing AP testing in the future (will still use it as a screening tool). Look into the cost of AP vs P30 testing 1/20 dilution will not be tested with this further testing

Update 31/07/2019: 1/20 dilution to be added to all scrapings. 1/20 dilution added and 1/500 discarded for all tape-lifts.

ss - Donor 4 - Thick fabric - Semen dil'n	Scraping Slide barcode	whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Sperm	Phadebas		
1/20	Silde barcode	whole sperm	<1+	<1+	neg	time (s)	pos	Dill silde barcode	<1+	neg		
1/20		<1+	<1+	<1+	neg		pos		2+	neg		
1/50		0	0	0	neg		pos		0	neg		
1/50		<1+	<1+	<1+	neg		pos		<1+	pos		
1/100		0	0	<1+	neg		pos		0	neg		
1/100		<1+	<1+	<1+	neg		pos		<1+	neg		
1/200		0	0	0	neg		pos		<1+	neg		
1/200 1/500		0	0	0 <1+	neg		neg		0	neg		
1/500		0	0	0	neg neg		neg neg		<1+	neg neg		
1/300	·	<u> </u>	<u> </u>	<u>~1</u>	псы	-	1108		``.	1		
ess - Donor 4 - Thick fab							us	se from 20/08				
Semen dil'n		Sperm	AP	time (s)	p30	Phadebas	1	7613-20190711_MA	M			microscopy:
1/20		<1+	neg		pos	pos	1	7751-23280702				HB better
1/20		1+	neg		pos	neg		r				
1/50		<1+	neg		NR*	neg		<u>-</u>			n a thermomixer	
1/50 1/100		1+	neg neg		pos neg	pos	*1	No result. P30 kit was fa	fulty (no contr	ol line came	up).	
1/100		<1+	neg		neg	neg pos						
1/200		1+	neg		pos	neg	1	7024-20190424_MA	м			
1/200		0	neg		neg	neg		7494-23280702				
1/500		0	neg		neg							
1/500		0	neg		neg	neg						
						3 - (1xHB, 2xT)						
ss - Donor 4 - Thin fabric			1				a - I	D.W. 2		SI		
Semen dil'n		whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Sperm	Phadebas		
1/20 1/20		<1+	<1+ <1+	1+ 2+	neg neg		pos pos		1+	neg neg		
1/50		0	0	<1+	neg		pos		<1+	neg		
1/50		0	0	<1+	neg		pos		<1+	neg		
1/100		0	<1+	1+	neg		pos		<1+	pos		
1/100		<1+	<1+	1+	neg		pos		0	neg		
1/200		0	0	1+	neg		neg		0	neg		
1/200		0	<1+	1+	neg		pos		<1+	neg		
1/500		0	0	<1+	neg		neg		<1+	neg		
1/500		0	<1+	<1+	neg		neg		0	neg		microsconu
ess - Donor 4 - Thin fabri										1		microscopy: HB and T data compar
Semen dil'n		Sperm	AP	time (s)	p30	Phadebas						no ana i data compan
1/20		1+	neg	- (-)	pos	neg						
1/20		1+	neg		pos	neg						
1/50		<1+	neg		pos	neg						
1/50		<1+	neg		pos	neg						
1/100		<1+	neg		pos	pos						
1/100		0	neg		neg	neg						
1/200 1/200		0	neg		neg	neg						
1/500		0	neg neg		neg neg	neg neg						
1/500		0	neg		neg	neg						
			-3		3xHB 2xT							
ss - Donor 5 - Thick fabric			1				a - I	D.((		<u> </u>		
Semen dil'n		whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Sperm	Phadebas		
1/20 1/20		0 <1+	<1+ <1+	<1+ <1+	neg		pos pos		1+ 1+	neg		
1/50		<1+	<1+	<1+	neg neg		pos*		0	neg pos	*Faulty p30 kit. An additional 150ul nanol	120 added to sample and p20 r
1/50		0	<1+	<1+	neg		neg		0	neg	radicy poor inc. All additional 15001 Hallot	.20 daded to sample and p30 I
1/100		0	<1+	1+	neg		pos		<1+	pos		
1/100		0	0	<1+	neg		neg		<1+	pos		
1/200		0	0	<1+	neg		neg		0	pos		
1/200		0	0	<1+	neg		neg		0	pos		
1/500		0	0	<1+	neg		neg		0	pos		
1/500		0	0	<1+	neg		neg		0	neg 6		
ess - Donor 5 - Thick fab										О		
Semen dil'n		Sperm	AP	time (s)	p30	Phadebas	us	se from 20/08				
1/20		<1+	neg	(5)	pos	neg		7613-20190711_MA	М			
1/20		2+	neg		pos	pos		7751-23280702				microscopy:
1/50		0	neg		neg	pos						HB and T data compa
1/50		0	neg		neg	neg		7024-20190424_MA	М			
1/100		<1+	neg		pos	neg	1	7494-23280702				
1/100		0	neg		pos	neg						
1/200 1/200		<1+ <1+	neg		neg	pos						
1/500		<1+	neg neg		neg neg	neg neg						
1/500		0	neg		neg	pos						
,		-1	6			4 (2xHB, 2xT)						
			_			-						

ocess - Donor 5 - 1					I						
	nen dil'n	Slide barcode	e whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Sperm	Phadebas
	1/20		<1+	1+	2+	neg		pos		1+	neg
	1/20		<1+	2+	2+	neg		pos		1+	neg
	1/50		0	<1+	1+	neg		pos		<1+	neg
	1/50 1/100		<1+ 0	0	<1+ 2+	neg		pos		<1+ <1+	neg
	1/100		0	0	1+	neg neg		pos neg		<1+	neg neg
	1/200		0	0	2+	neg		neg		0	neg
	1/200		0	0	1+	neg		neg		0	neg
	1/500		0	0	2+	neg		neg		<1+	neg
	1/500		0	0	1+	neg		neg		0	neg
ess - Donor 5	- Thin fa	hric - Scr									0
	nen dil'n	Diff	Sperm	AP	time (s)	p30	Phadebas				
	1/20		1+	neg		pos	neg				
	1/20		1+	neg		pos	neg				
	1/50		0	neg		pos	neg				
	1/50 1/100		<1+ 0	neg		pos neg	neg				
	1/100		<1+	neg neg		pos	neg neg				
	1/200		<1+	neg		neg	neg				
	1/200		0	neg		neg	neg				
	1/500		0	neg		neg	neg				
	1/500		<1+	neg		neg	neg				
						xHB 3xT	0				
s - Volunteer		fabric -									
Sem	nen dil'n		whole sperm	sperm heads	epithelials	AP	time (s)	p30	Diff Slide barcode	Sperm	Phadebas
	1/20		0	<1+	<1+	neg		pos		2+	neg
	1/20 1/50		0	<1+ <1+	<1+ 0	neg		pos		3+ <1+	neg neg
	1/50		0	<1+	<1+	neg neg		pos		<1+	pos
	1/100		0	<1+	<1+	neg		neg		0	neg
	1/100		0	0	<1+	neg		neg		<1+	pos
	1/200		0	<1+	0	neg		pos		1+	pos
	1/200 1/500		0	<1+	0	neg		pos		1+ 0	pos
	1/500		0	0	<1+ <1+	neg neg		neg neg		0	neg
	,					-01		- 0			
											5
ess - Volunte											3
	nen dil'n	ck fabric Diff	Sperm	AP	time (s)	p30	Phadebas	Nov	. hatabaa	•	5
	nen dil'n 1/20		<1+	neg	time (s)	neg	neg		v batches:	NA.	3
	1/20 1/20		<1+ 1+	neg neg	time (s)	neg pos	neg neg	176	13-20190711_MA	ıM	3
	nen dil'n 1/20		<1+	neg	time (s)	neg	neg	176		м	3
	1/20 1/20 1/20 1/50		<1+ 1+ 2+	neg neg neg	time (s)	neg pos pos	neg neg neg	176	13-20190711_MA	м	3
	1/20 1/20 1/50 1/50 1/100 1/100		<1+ 1+ 2+ 3+ 1+ 0	neg neg neg neg neg	time (s)	neg pos pos pos pos neg	neg neg neg pos pos	17 <i>6</i> 177 use	513-20190711_MA 750-23280516 from 20/08		3
	1/20 1/20 1/50 1/50 1/100 1/100 1/200		<1+ 1+ 2+ 3+ 1+ 0	neg neg neg neg neg neg	time (s)	neg pos pos pos pos neg pos	neg neg neg pos pos neg pos	176 177 use 176	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA		5
	1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/200		<1+ 1+ 2+ 3+ 1+ 0 0 <1+	neg neg neg neg neg neg neg neg neg	time (s)	neg pos pos pos pos pos neg pos neg	neg neg neg pos pos neg pos neg	176 177 use 176	513-20190711_MA 750-23280516 from 20/08		5
	1/20 1/20 1/50 1/50 1/100 1/100 1/200		<1+ 1+ 2+ 3+ 1+ 0	neg	time (s)	neg pos pos pos pos neg pos	neg neg neg pos pos neg neg neg	176 177 use 176	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA		3
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500 1/500	Diff	<1+ 1+ 2+ 3+ 1+ 0 <1+ 0 <1+	neg neg neg neg neg neg neg neg neg		neg pos pos pos pos neg pos neg pos neg	neg neg neg pos pos neg neg neg neg	176 177 use 176	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA		3
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/50 1/100 1/200 1/200 1/500 1/500	Diff	<1+ 1+ 2+ 3+ 1+ 0 <1+ 0 <1+ 0 0 <1+ 0 0 <1+ 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	neg		neg pos pos pos neg pos neg pos neg pos AxHB 3xT 3	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	м	
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff	<1+ 1+ 2+ 3+ 1+ 0 <1+ 0 <1+ 0 whole sperm	neg	epithelials	neg pos pos pos neg pos neg pos AxHB 3xT 3	neg neg neg pos pos neg neg neg neg	176 177 use 176 177	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA	. <b>M</b> Sperm	Phadebas
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff	<1+	neg	epithelials	neg pos pos pos neg pos neg pos AxHB 3xT 3	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+	Phadebas neg
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff	<1+ 1+ 2+ 3+ 1+ 0 <1+ 0 <1+ 0 whole sperm	neg	epithelials	neg pos pos pos neg pos neg pos AxHB 3xT 3	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	. <b>M</b> Sperm	Phadebas
Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/50	Diff	<1+	neg	epithelials 1+ 2+	neg pos pos pos pos neg pos neg pos AXHB 3XT 3	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177 177	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+	Phadebas neg neg
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff	<1+	neg	epithelials 1+ 2+ <1+ 1+ <1+	neg pos pos pos neg pos neg pos AXHB 3XT 3  AP neg neg neg neg neg neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177  177  p30 pos pos pos pos pos pos	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 1+ <1+	Phadebas neg neg pos neg
Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff	<1+	neg	epithelials 1+ 2+ <1+ 1+ <1+ 1+	neg pos pos pos neg pos pos pos AXHB 3XT 3  AP neg neg neg neg neg neg neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177  177  p30 pos pos pos pos pos pos pos	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 1+ <1+ 0	Phadebas neg neg pos neg neg
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/200 1/500 1/200	Diff	<1+	neg	epithelials 1+ 2+ <1+ 1+ <1+ 1+ 1+	neg pos pos pos neg pos pos pos AXHB 3XT 3  AP neg neg neg neg neg neg neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177  p30 pos pos pos pos pos pos pos pos	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 1+ <1+ 0 <1+	Phadebas neg neg pos neg neg neg
Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/20 1/50 1/20 1/50 1/20 1/50 1/50 1/50 1/100 1/200 1/200	Diff	<1+	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+	neg pos pos pos neg pos pos pos AxHB 3xT 3  AP neg neg neg neg neg neg neg neg neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	p30 pos pos pos pos pos pos neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ <1+ 0 <1+ <1+ <1+	Phadebas neg neg pos neg neg neg
Sem	nen dil'n 1/20 1/20 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/200 1/500 1/200	Diff	<1+	neg	epithelials 1+ 2+ <1+ 1+ <1+ 1+ 1+	neg pos pos pos neg pos pos pos AXHB 3XT 3  AP neg neg neg neg neg neg neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	176 177 use 176 177  p30 pos pos pos pos pos pos pos pos	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 1+ <1+ 0 <1+	Phadebas neg neg pos neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500 1/500	Diff  fabric - S	<1+	neg	epithelials 1+ 2+ <1+ 1+ 2+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+	neg pos pos pos pos neg pos pos pos AXHB 3XT 3  AP neg	neg neg neg pos pos neg pos neg pos neg pos neg neg neg neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500	fabric - S	<1+	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos neg pos neg pos pos AxHB 3xT 3  AP neg	neg neg neg neg pos pos neg pos neg neg (2xHB, 1xT)	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/200 1/500	Diff  fabric - S	<1+ 1+ 2+ 3+ 1+ 0 0 <1+ 0 <1+ 0 0 <1+ 0 0 <1+ 0 0 <1+ 0 0 <1+ 0 0  <1+ 0 0  Sperm	neg	epithelials 1+ 2+ <1+ 1+ 2+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+	neg pos pos pos neg pos neg pos pos AxHB 3xT 3  AP neg	neg neg neg neg pos pos neg pos neg neg neg reg neg neg neg neg neg neg neg neg neg n	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500	fabric - S	<1+	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos neg pos neg pos pos AxHB 3xT 3  AP neg	neg neg neg neg pos pos neg pos neg neg neg neg neg neg neg neg neg (2xHB, 1xT)	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500	fabric - S	<pre></pre>	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos neg pos pos pos pos AXHB 3XT 3  AP neg	neg neg neg neg pos pos neg pos neg neg neg reg neg neg neg neg neg neg neg neg neg n	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500	fabric - S	<pre></pre>	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos pos neg pos	neg neg neg neg neg pos pos neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500	fabric - S	<pre></pre>	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos system pos	neg neg neg neg neg pos pos neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/100 1/100 1/200 1/500	fabric - S	<pre></pre>	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos pos neg pos neg pos pos AXHB 3XT 3  AP neg	neg neg neg neg neg pos pos neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/200 1/500	fabric - S	<pre></pre>	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos suppos pos pos pos pos pos pos pos pos pos	neg neg neg neg neg pos pos neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500	fabric - S	Call   Call	neg	epithelials 1+ 2+ <1+ 1+ 1+ 1+ 2+ 2+ 1+ 2+	neg pos pos pos pos neg pos neg pos pos neg pos neg pos pos axHB 3xT 3  AP neg	neg neg neg neg neg pos pos neg pos neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg
s - Volunteer Sem	nen dil'n 1/20 1/50 1/50 1/50 1/100 1/100 1/200 1/500 1/500 1/500 1/500 1/200 1/500 1/200 1/500 1/200 1/500 1/200 1/500 1/200 1/500 1/200 1/500	fabric - S	Call   Call	neg	epithelials 1+ 2+ <1+ 1+ 2+ 1+ 2+ time (s)	neg pos	Phadebas neg neg neg neg neg nes neg	p30 pos pos pos pos pos neg neg neg	513-20190711_MA 750-23280516 from 20/08 513-20190711_MA 751-23280702	Sperm 4+ 3+ 1+ 2+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1+ <1	Phadebas neg neg pos neg neg neg neg neg

Current process - Volunteer 2 - Thick fabric - Scraping

microscopy: Thermo is better

microscopy: HB is better

microscopy: HB and T are comparable

Proceedings   State Name   State State   Sta								
1.02			Slide barcode whole sperm		epithelials		time (s)	p30 Diff Slide barcode Sperm Phadebas
1,15    0   0   0   0   1,76    1,76								
17.72						-0		
1.200						_ ~		
1.700								
1,000   10   0   0   0   0   0   0   0   0						<u> </u>		<u> </u>
1,000   0   0   0   0   0   0   0   0   0				<b>.</b>				
2500   0   0   1-1   mg   mg   mg   mg   mg   mg   mg   m								
Proposed process   Proposed pr								<u> </u>
Proposed process. Voluntees 2 - Thois 1		_						
Second process - Volunteer 2 - This Form of Col.   Second process - Volunteer 2 - This East   Second process	1,30	<u> </u>			1	псы		
Security	Proposed process - Volunteer 2 - T	hick f						<u> </u>
1.10    1   1   1   1   1   1   1   1   1			Sperm	ДР	time (s)	n30	Phadehas	
102   1   102   103   105   104   105   105   104   105   104   105   104   105   104   105   104   105   104   105   104   105   105   104   105   10				<b>†</b>	` '			
1.10				<u> </u>				1
1.750				<del> </del>				
1,700		_	<1+					
1,700		_		<u> </u>				
1/200								1
1/30	1/20	0	<1+					1
1756		_						
Current process - Volunteer 2 - This factors   Septem Process   Septem P	-							
Current process - Volunteer 2 - Thin fabr   Series (CT)	-	_						1
Service of the content of the cont			'					•
Sement distance   Sement dis	Current process - Volunteer 2 - Th	in fabr				'1	v <del>-</del>	
1700			whole sperm	sperm heads	epithelials	AP	time (s)	p30 <u>Diff Slide barcode</u> Sperm Phadebas
170	1/2	0	•	<u> </u>				
1/50			- <del>1</del>					-
1,150								
1/100	-	_						
1,700						1		
1/200				0				
1/500	1/20	0		<1+	1+			pos 0 neg
1/500	1/20	0		0	1+	neg		neg 0 neg
Proposed process - Volunteer 2 - Thin fa Sample D Semen diffil Sperm AP time (s) p30 Phadebas   1/70   11 neg   p00 neg   1/70   12 neg   p00 neg   neg   1/700   12 neg   p00 neg   neg   1/700   12 neg   p00 neg   neg   1/700   13 neg   p00 neg   neg   neg   p00 neg   neg   p00 neg   neg   p00 neg   p	1/50	0		0	1+			
Proposed process - Volunteer 2 - Thin fa  Sample D  Semend If n  Semend If n  Semend If n  1/20  12	1/50	0		0	1+	neg		neg <1+ neg
Semolar ID   Semon of   Semon o			•	•	•			
1/70 1/76 1/76 1/76 1/76 1/76 1/76 1/76 1/76	Proposed process - Volunteer 2 - T	hin fa						
1/50	Sample ID Semen dil	n	Sperm	AP	time (s)	p30	Phadebas	
1/50	1/2	0	1+	neg		pos	neg	
1/1/10	1/2	0	<1+	neg		pos	neg	
1/100	1/5	0	<1+	neg		pos	neg	
1/100	1/5	0	<1+	neg		pos	neg	
1/200	1/10	0	<1+	neg		neg	neg	
1/500	1/10	0		neg		neg	neg	
1/500	1/20	0	<1+	neg		pos	neg	
Current process - Volunteer 3 - Thick fab   Sample ID   Semen diff   Whole sperm   Sperm heads   epithelials   AP   time (s)   p.30   Diff Slide barcode   Sperm   Phadebas	1/20	0	(	neg		neg	neg	
Sample   D   Semendil's   Sem	1/50	0	C	8		neg	neg	
Current process - Volunteer 3 - Thick fab	1/50	0		neg		neg	neg	
Sample   D   Semen diff						3xHB 2xT	0	
Sample   D   Semen diff								
1/20								
1/20							time (s)	
1/50				<u> </u>				
1/50						<u> </u>		
1/100						-0		
1/100		_				- 1		
1/200								<u> </u>
1/200						<del>                                     </del>		
1/500   0   0   1/4   neg   neg   0   pos   microscopy: HB and T comparat		_						
1/500						1		
Sample ID   Semen dil'n   Sperm   AP   time (s)   p30   Phadebas   17613-20190711_MAM   1750-23280516   1750   14   neg   pos   neg   17613-20190711_MAM   1750-23280516   1750   14   neg   pos   neg   17024-20190424_MAM   1750-23280516   17613-20190711_MAM   1750-23280516   17613-20190711_MAM   1750-23280516   17613-20190711_MAM   1750-23280516   17613-20190711_MAM   1750-23280516   17613-20190711_MAM						<del> </del>		
Proposed process - Volunteer 3 - Thick f   Sample ID   Semen dil'n   Sperm   AP   time (s)   p30   Phadebas   1/20   1+   neg   pos   neg   1/50   1+   neg   pos   neg   1/50   1/50   1+   neg   pos   neg   1/50   1/5	1/50	υ	Ţ	<u> </u>	<1+	neg		
Sample ID   Semen dil'n   Sperm   AP   time (s)   p30   Phadebas   New batches:   17613-20190711_MAM   17750-23280516     1750	Dunmand	hiele e						3
1/20					A* / 3	-201	Nhl - l-	Now hatches:
1/20		_		<b>†</b>	time (s)			
1/50								
1/50			_					
1/100								1
1/100		_		· · · · ·				
1/200								
1/200				<del> </del>				1
1/500 0 neg neg pos 4xHB 3xT 2 (1xHB, 1xT)  Current process - Volunteer 3 - Thin fabric - Scraping  Sample ID Semen dil'n Slide barcode whole sperm sperm heads epithelials AP time (s) p30 Diff Slide barcode Sperm Phadebas 1/20 4xHB 3xT 2 (1xHB, 1xT)  17751-23280702		_						
1/500		_		<u> </u>				
AxHB 3xT 2 (1xHB, 1xT)  Current process - Volunteer 3 - Thin fabric - Scraping  Sample ID Semen dil'n Slide barcode whole sperm sperm heads epithelials AP time (s) p30 Diff Slide barcode Sperm Phadebas  1/20		_	·	<u> </u>				
Current process - Volunteer 3 - Thin fabric - Scraping  Sample ID Semen dil'n Slide barcode whole sperm sperm heads epithelials AP time (s) p30 Diff Slide barcode Sperm Phadebas  1/20 <	1/50	<u> </u>	1 <14	neg	I .			I
Sample ID Semen dil'n Slide barcode whole sperm sperm heads epithelials AP time (s) p30 Diff Slide barcode Sperm Phadebas  1/20 <	Current process - Volunteer 2 Thi	n fahr!	c - Scraning			4XIID 3XI	Z (IXIID, IXI)	
1/20 <1+ 1+ 1+ neg pos 3+ neg				cnarm hands	onitholiala	ΛĐ	time (c)	n30 Diff Slide harcode Sperm Dhadobas
							time (S)	
1 11 11 11Eg PU3								
	1/2	~			1+	IIIC S		2. 1105

microscopy: HB is better

microscopy: HB is better

# Phadebas results:

Current process better:
Proposed process better:
No difference:
Positive thermomixer:
Positive heatblock:

# Microscopy results:

Vol 1 – thick: Proposed process appears better than the current process and better than the diff slide process
Vol 1 – thin: Proposed process appears consistent with the current process but worse than the diff slide process
Vol 2 – thick: Proposed process appears better than the current process and consistent with the diff slide process
Vol 2 – thin: Proposed process appears consistent with the current process but worse than the diff slide process
Vol 3 – thick: Proposed process appears better than the current process and better than the diff slide process
Vol 3 – thin: Proposed process appears consistent with the current process but worse than the diff slide process

	Ī						
1/50	0	<1+	1+	neg	pos	<1+	neg
1/50	0	1+	1+	neg	pos	<1+	neg
1/100	0	0	1+	neg	pos	<1+	neg
1/100	0	<1+	1+	neg	pos	<1+	neg
1/200	0	0	<1+	neg	neg	<1+	neg
1/200	0	<1+	1+	neg	pos	<1+	neg
1/500	0	0	1+	neg	neg	<1+	neg
1/500	0	0	1+	neg	neg	<1+	neg
							•

Proposed process - Volunteer 3 - Thin fabric - Scraping

_	oposca proc	tess - volunteer 5 - III	III IUDII	c scraping					
	Sample ID	Semen dil'n	Di	ff Slide barcode	Sperm	AP	time (s)	p30	Phadebas
		1/20			2+	neg		pos	neg
		1/20			1+	neg		pos	neg
		1/50			<1+	neg		pos	neg
		1/50			<1+	neg		neg	neg
		1/100			<1+	neg		pos	neg
		1/100			<1+	neg		neg	neg
		1/200			0	neg		neg	neg
		1/200			<1+	neg		neg	neg
		1/500			0	neg		neg	neg
		1/500			0	neg		neg	neg
								3xHB 1xT	0

microscopy: HB and T comparable Donor 4 – thick: Proposed process appears better than the current process but worse than the diff slide process

Donor 4 – thin: Proposed process appears worse than the current process and worse than the diff slide process

Donor 5 – thick: Proposed process appears better than the current process and better than the diff slide process

Donor 5 – thin: Proposed process appears better than the current process and consistent with the diff slide process

mermomixer better:	1
Heatblock better:	4
No difference:	5
Proposed process better than current process	6
Proposed process consistent with current process	3
Proposed process worse than current process	1
Proposed process better than diff slide	3
Proposed process consistent with diff slide	2
Proposed process worse than diff slide	5

Discussed results with management and decided on next steps: Test excisions, tapelifts and swabs with 3 x donors. Donor 4, volunteer 1 and volunteer 3 should be used (as these gave the best results for this stage of testing) 1/20, 1/50, 1/100, 1/200, 1/500 are to be done for all test types

All others factors to remain the same

Current process - Donor 4 - Thick feb	oric - Tape-lift						Did extra H2O have to be added to the p30 kit?
Sample ID   Semen dil'n   1/20   1/20	Slide harcode whole sperm 0	sperm heads 0	epithelials 0	AP neg neg	time (s)	p30 Diff Side barcode Sperm Phadebas  neg 0 neg  neg <1+ neg	No No
1/50	0	0	0 d+	neg		neg de neg	No No
1/100 1/100 1/200	0	0	0	neg neg		neg 0 neg	No No No
1/200 1/500 1/500	0	0	0	neg neg		neg         <1+         neg           neg         0         neg           neg         0         neg	No No No
Proposed process - Donor 4 - Thi	- 0		· ·	neg		neg 0	
Semen dil'n 1/20	Sperm <1+	AP neg	time (s)	p30 neg	Phadebas neg		No No
1/20 1/50 1/50	de   de   de	neg neg		neg neg	neg neg	17613-20190711_MAM 18388-23290311	No No No
1/100 1/100	<1+ <1+ 0	neg neg		neg	neg neg		No
1/200 1/200 1/500	0	neg neg		neg neg	neg neg		No No No
1/500	0	neg		neg	neg O		No
Current process - Donor 4 - Thin 1 Semen dil'n 1/20	whole sperm	sperm heads 0	epithelials 0	AP neg	time (s)	p30 Diff Slide barcode Sperm Phadebas neg 0 neg	No
1/20	0	0	0	neg		neg <1+ neg neg 0 neg	No No
1/50 1/100 1/100	0	0	0	neg neg		neg 0 neg neg 0 neg neg 0 neg	No No No
1/200	0	0	0	neg		neg 0 neg	No
1/500 1/500	0	0	<1+ 0	neg		neg 0 neg 0 neg 0	No No
Proposed process - Donor 4 - Thi Sample ID Semen dil'n 1/20	Sperm 1+	AP	time (s)	p30	Phadebas	17613-20190711_MAM	No
1/20 1/50	0	neg neg		neg neg neg	neg neg	18388-23290311	No No No
1/50	0	neg neg		neg	neg neg		No No No
1/100 1/200 1/200	0	neg neg		neg neg neg	neg neg	19377-20200109KA 19228-23290924	No No
1/500 1/500	0	neg neg		neg	neg		No No
Current process - Volunteer 1 - T							_
Semen dirn	whole sperm 0	sperm heads 0	epithelials 0	AP neg	time (s)	p30 Diff Slide barcode Sperm Phadebas neg <1+ neg	No
1/20 1/50 1/50	0	0	0	neg neg neg		neg         1+         neg           neg         <1+	No No No
1/100	0	0	0	neg		neg 0 neg	No No
1/200 1/200 1/500	0	0	0 0 <1+	neg neg		neg 720055043 <1+ neg neg 0 neg	No No Yes
1/500	0	0	0	neg		neg 0 neg	Yes
Proposed process - Volunteer 1 - Sample ID Semen dil'n 1/20	Sperm 1+	AP neg	time (s)	p30 neg	Phadebas neg		No
1/20	<1+	neg		neg	neg neg	17613-20190711_MAM 18388-23290311	No No
1/50 1/100 1/100	0	neg neg		neg neg	neg neg neg		No No No
1/200 1/200	0	neg		neg	neg neg		Yes No
1/500 1/500	0	neg		neg	neg neg 0		No No
Current process - Volunteer 1 - T Sample ID Semen dil'n	whole sperm	sperm heads	epithelials	AP	time (s)	p30 Diff Slide barcode Sperm Phadebas	No
1/20 1/20 1/50	0	0	0	neg neg		neg de neg	No No
1/50 1/100 1/100	0	0	0	neg		neg 1e neg	No No No
1/200 1/200	0	0	1+	neg neg		neg de neg	No No
1/500 1/500	0	0	0	neg		neg 0 neg 0 neg	No No
Proposed process - Volunteer 1 - Semen dil'n	Sperm	AP	time (s)	p30	Phadebas	v	
1/20 1/20 1/50	1+ 	neg neg neg		neg neg	neg neg neg	17613-20190711_MAM 18388-23290311	No No
1/50	0	neg neg		neg	neg		No No
1/100 1/200 1/200	d+	neg neg neg		neg neg	neg neg	19377-20200109KA	No No No No No No
1/500 1/500	<1+ <1+ 0	neg		neg	neg	19228-23290924	No No
Volunteer 3 - T					0		_
Semen dil'n	0	sperm heads 0	epithelials 0	AP	time (s)	p30 Diff Slide barcode Sperm Phadebas neg <1+ neg	No No
1/20 1/50 1/50	0	0	0	neg neg		neg         <1+         neg           neg         0         neg           neg         <1+         neg	No No No
1/100	0	0	0	neg		neg 0 neg neg 0 neg	No No No
1/200 1/200 1/500	0	0	0	neg		neg 0 neg neg de neg	No No
1/500 Proceed process - Volunteer 3 -	0	0	0	neg		neg 0 neg	No
Semen dil'n	Sperm 0 0	AP neg	time (s)	p30 neg	Phadebas neg		Yes
1/20 1/50 1/50	0	neg neg neg		neg neg	neg neg	17613-20190711_MAM 18388-23290311	No No
1/100	0	neg neg		neg	neg neg		No No No No
1/200 1/200 1/500	0	neg neg		neg neg	neg neg neg		No No No
1/500	0	neg		neg	neg 0		No
Volunteer 3 - T Semen dil'n 1/20	whole sperm	sperm heads	epithelials 0	AP	time (s)	p30 Diff Slide barcode Sperm Phadebas neg <1+ neg	No
1/20	0	0	0	neg		neg cle neg o neg	No No
1/50 1/100 1/100	0	0	0 <1+	neg neg		neg 0 neg 0 neg neg 1 ne	No No No
1/200 1/200	0	0	0	neg		neg de neg	No No No No
1/500 1/500	0	0	0	neg		neg 0 neg 0 neg 0	No No
						•	

1/20 1/20 1/50 1/50 1/100	Diff Slide barcode Sperm	AP	time (s)	p30	Phadebas		
1/50	de de	neg neg		neg neg	neg	17613-20190711_MAM 18388-23290311	No No No No No No No No
	<1+	neg		neg	neg neg		No No
1/100	0	neg neg		neg neg	neg		No
1/200 1/200	<1÷	neg		neg neg	neg neg	19377-20200109KA	No No
1/500 1/500	0	neg neg		neg neg	neg	19228-23290924	No
December 1981					0		
- Donor 4 - Thick fab Semen dil'n 1/20	nole sperm	sperm heads	epithelials 1+	AP	time (s) 1 minute	p30 Diff Slide barcode Sperm Phadebas pos 1+ pos	Yes
1/20 1/50	0	<1+	0	pos	1 minute	pos 1+ pos	Yes
1/50 1/100	<1+	10	ds 10	neg neg neg		pos 1+ pos pos 3+ pos pos 1+ pos	Yes Yes
1/100 1/200	0	<1+	d+	neg		pos 1+ pos (1+ pos	Yes Yes
1/200 1/500	0	0	1+	neg neg		pos 1+ pos pos 0 pos	Yes Yes
1/500	0	0	0	neg		pos	Yes
ss - Donor 4 - Thick fo Semen dil'n		AP	time (s)	p30	Phadebas		Yes
1/20	2+	pos pos	40 seconds 1 minute and 30 seconds	pos pos	pos pos	AP: 17613-20190711_MAM	No Yes
1/50 1/50	2+	neg pos	2 minutes	pos pos	pos pos	P30: 17860-23280702	No No
1/100 1/100	1+	neg		pos pos	pos	new p30: 18388-23290311	No No No No
1/200 1/200 1/500	d+ d+	neg		pos	pos	18388-23290311	No No
1/500	d+	neg		pos pos	pos pos 10 (SxHB SXT)		No No
- Donor 4 - Thin fabr Semen dil'n	ic - Excision Slide barcode whole sperm	sperm heads	epitheliais	AP		p30 Diff Slide barcode Sperm Phadebas	
4/30 The	e samples were left at room tempera therefore are not suitable for analysis	ture over the we		906	45 seconds	906	Yes Yes
1/50 All s	amples to be reprepared and resample	led using the bard	codes below	900	1 minute	900	Yes No
1/100 1/100				pos		906	Yes Yes
4/300 1/300						pec	No Yes
4/000 1/600				700g		pos	Yes
1/20	0	<d+< td=""><td>10</td><td>pos pos</td><td>1 minute 1 minute and 15 secs</td><td>pos se pos se po</td><td>No No</td></d+<>	10	pos pos	1 minute 1 minute and 15 secs	pos se po	No No
1/50 1/50 1/100	0	0 <1+ 1+	d+ d+	neg		pos   2+   pos	No No No
1/100 1/100 1/200	0	<1+	d+	neg neg neg		pos <1+ pos	No No No
1/200 1/500	0	<1+	d+	neg		pos	No No
1/500	0	0	1+	neg		neg 0 pos 10 (SxHB SXT)	No No
ss - Donor 4 - Thin fa						19377-20200109KA	
Semen dil'n 1/20	Sperm 1+	AP neg	time (s)	p30 pos	Phadebas pos	18388-23290311	No
1/20 1/50 1/50	1+	neg		pos pos	pos	17613-20190711_MAM	No No No No No No No No
1/100	1+	neg		pos	pos	18388-23290311	No No
1/100 1/200 1/200	1e <1e <1e	neg neg		pos pos pos	pos pos pos		No No
1/500 1/500	d+	neg		pos pos	pos		No No
					10 (5xHB 5XT)		
ss - Volunteer 1 - Thick  Semen dil'n	whole sperm	sperm heads	epithelials	AP	time (s)	p30 Diff Slide barcode Sperm Phadebas	Yes
4 1/20 6 1/20	0 <1+	10	0	pos pos	40 seconds 1 minute 2 minutes	pos 3+ pos 90	Yes Yes
5 1/50 9 1/50 8 1/100	0	<d+ <d+< td=""><td>1+ 1+</td><td>pos</td><td>1 minute 40 seconds</td><td>pos &lt;1+ pos</td><td>Yes Yes Yes</td></d+<></d+ 	1+ 1+	pos	1 minute 40 seconds	pos <1+ pos	Yes Yes Yes
7 1/100 1 1/200	0	0	1+	neg neg		pos   1 e   pos	Yes Yes
0 1/200 4 1/500	0	<1+	1+	neg		pos 1+ pos	
				neg		pos d+ pos	Yes
3 1/500	<i+< td=""><td>de</td><td>1+</td><td>neg</td><td></td><td></td><td>Yes Yes Yes</td></i+<>	de	1+	neg			Yes Yes Yes
cess - Volunteer 1 - Thi	<1+ Sperm	AP	time (s)	neg p30	Phadebas	pos	Yes Yes Yes
3 1/500 cess - Volunteer 1 - Thi D Semen dil'n 2 1/20 7 1/20	Sperm 3+ 3+	AP pos pos		p30 pos pos	pos pos	pos	Yes Yes Yes
3 1/500  cess - Volunteer 1 - Thi  Semen dirn  2 1/20  7 1/20  6 1/50  5 1/50	Sperm 3+ 3+ <1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1	AP pos pos neg	time (s) 1 minute and 15 seconds	p30 pos pos pos	pos pos pos	pos	Yes Yes Yes
3 1/500 cess - Volunteer 1 - Thi 5 Semen dil'n 2 1/20 7 1/20 6 1/50	Sperm 9e 3e 11e 11e 11e 11e 11e 11e 11e 11e 11e	AP pos pos neg	time (s) 1 minute and 15 seconds	p30 pos pos pos pos pos	pos pos pos pos pos	pos	Yes Yes Yes
3 1,500 coss - Volumer 3 - Thi D Samen dTn D Samen dTn D 1,700 7 1,700 5 1,700 8 1,700 1 1,700 1 1,700 0 1,700 0 1,700 0 1,700 0 1,7500 0	Sperm 38 38 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	AP posi posi neg neg neg neg	time (s) 1 minute and 15 seconds	p30 pos pos pos pos	905 905 905 905 905 905 905 905	Pos	Yes Yes Yes No
5	Sperm 30 30 31 31 32 32 32 32 32 33 33 33	AP pos pos neg neg neg neg neg	time (s) 1 minute and 15 seconds	p30 pos pos pos pos pos pos pos	pos pos pos pos pos pos	POS	Yes Yes Yes
5	Sperm 30 30 31 31 32 32 32 32 32 33 33 33	AP pos pos nes nes nes nes nes nes nes nes ses se	time (s)  1 minute and 15 seconds  45 seconds  epithelials	p30 pos pos pos pos pos pos pos pos	pos   time (s)	Pos	Yes Yes Yes No
a) \$2,000   coss Volunter 1 Th  ) Semen (If n)  2	Sperm 3a	AP pos pos neg	time (s)  1 minute and 15 seconds  45 seconds  epithelials	p30 pos	pos    Pos	Yes Yes No Yes Yes	
as Volunteer 1 TM beams 6ffn 2 1/20 7	Sperm   3a   3a   3a   3a   3a   3a   3a   3	AP pos pos neg	time (s)  1 minute and 15 seconds  45 seconds  epithelials	930 pos pos pos pos pos pos pos pos pos pos	pool pos	Pos	Yes Yes No No No No No No No No No Yes Yes Yes
2,500	Sperm 3a	AP pos pos neg	time (s)  1 minute and 15 seconds  45 seconds  epithelials	930 pos	pool	Pos. 1-1 pos 1	Yes Yes No
as Volunteer 1 TM beams 6ffn 2 1/20 7	Sperm 3a	AP pos pos neg	time (s)  1 minute and 15 seconds  45 seconds  epithelials	P30 P05 P05 P05 P05 P05 P05 P05 P05 P05 P0	pool	Pos. 1-1 pos 1-2 (0.149 SXT)  AP: 17613-20190711_MAN P20: 17660-32280702  new p30: 18388-32290311  p20 Diff Side harcode Sparm Phadebas pse 1-2 pos 1-	Yes Yes No Yes Yes Yes Yes Yes Yes Yes Yes
a) 1,000 ons - Volunter 1 Th  3 Semen 6f h  4 1,700 5 1,700 6	Spem 38 38 38 31 31 31 32 32 32 34 35 34 35 36 36 36 36 36 36 36 36 36 36 36 36 36	AP  pool  pool  neg  neg  neg  neg  neg  neg  span  neg  neg  tel  span  neg  tel  span  s	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  epithelials  codes below	p30   p05   p06   p06	P00    Pos	Yes Yes Yes No Yes	
a) 1,500 coss Voluntear 1 Th  ) Semen 6f n  2 1,200  1,200  1,200  1,100  1,100  1,100  1,100  1,100  1,100  2,100  1,100  1,100  2,100  2,100  2,100  2,100  3,100  3,100  4,100  5,100  5,100  6,100	Sperm 38 38 31 31 32 32 32 32 34 32 34 35 36 36 36 36 36 36 36 36 36 36 36 36 36	AP point poi	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  skend  codes below	p30	pool	Pos	Yes Yes Yes No Yes Yes Yes Yes Yes Yes Yes Yes No
a) \$\( \frac{1}{2}\) (000)  ones Volunter 1 Th  ) Semen (if \) (2  1 \( \frac{1}{2}\) (2  5	Sperm 38 38 38 31 31 32 32 32 32 34 32 34 35 36 36 386 barcode whole sperm 386e barcode whole sperm 386e barcode and resample of the sperm of the sp	post post post post post post post post	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  epithelials  codes below	p30   p30	P00    Pos	Yes Yes Yes No Yes Yes Yes Yes Yes Yes Yes Yes No	
a) 4/500   coss - Volunter 1 - Th   d) Seman 6f'n   2	Sperm 38 38 31 31 32 32 32 32 34 32 34 32 34 32 34 32 34 32 34 34 34 34 34 34 34 34 34 34 34 34 34	AP post post post post post post post post	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  epithelials  codes below  42  44  45  45  45  45  45  45  45  45	p30   p50   p50	P00    Pos	Yes Yes Yes Yes No	
a) \$2,000 ones Volunter 1 Thi  D Semen GFn 2 1/20 7 1/20 5 1/20 6 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 2 1/200 2 1/200	Sperm 38 38 38 38 38 38 38 38 38 38 38 38 38	AP post post post post post post post post	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  extend  codes bellow  C1+  C2+  C3+  C3+  C4+  C4+  C4+  C4+  C4+  C4	p30   p50   p50	pool	POS. 1-1 pos 1-2 pos 1	Yes Yes Yes Yes No
2,000   1,00	Sperm 3a	AP post post post post post post post post	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epitheliels  skand  codes below  C1s  C2s  C2s  C3s  C4s  C4s  C4s  C4s  C4s  C4s  C4	post	pool	POS	Yes Yes Yes Yes No
a) \$2,000 ones Volunter 1 Thi  D Semen GFn 2 1/20 7 1/20 5 1/20 6 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 1 1/20 2 1/200 2 1/200	Sperm 38 38 38 38 38 38 38 38 38 38 38 38 38	AP Pool pool pool pool pool pool pool pool	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  extend  codes below  C1+  C2+  C3+  C3+  C4+  C4+  C4+  C4+  C4+  C4		pos	Post	Yes Yes Yes Yes No
a) \$2,000   coss Volunter 1 Th  ) Semen (if n)  2 1/20  5 1/20  6 1/20  6 1/20  7 1/20  1 1/20	Sperm 3e	AP pool pool pool pool pool pool pool poo	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  related to the condess below  1 minute and 15 seconds  epithelials  epitheli	100   100	pool	Pos. 1-1 pos 1-2 pos 1-2 (2.149 SST)  AP: 17913-20190711 MAN P20: 17960-23280702  new p30: 18388-23290311  Page 1 pos 1	Yes Yes Yes No
a) \$\( \frac{1}{2}\) (000)  cons - Volunter 1 Th  )   Seman 6fr    2	Sperm 3a	AP point poi	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  related to the condess below  1 minute and 15 seconds  epithelials  epitheli	100   100	pool	Posi	Yes Yes Yes No
a) 1,000 ones - Voluntera 1 Th  ) Semen 6fr 1 2 1,720 5 1,720 5 1,720 6 1,720 7 1,720 6 1,720 7 1,720	Sperm 38 38 38 38 38 38 38 38 38 38 38 38 38	AP post post post post post post post post	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  related to the condess below  1 minute and 15 seconds  epithelials  epitheli	Page	peace   peac	POS	Yes Yes Yes No
a) \$\ \( \frac{1}{2}\) \( \cdot \) \(\cdot \) \( \cdot \) \( \cdot \) \( \cdo \) \( \cdot \) \( \cdot	Sperm 38 38 38 38 38 38 38 38 38 38 38 38 38	Sparm heads for the week of the heads for the week of the heads for the week of the heads for the he	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  related to the condess below  1 minute and 15 seconds  epithelials  epitheli	Page	Post	POS	Yea Yea No
a) \$\( \frac{1}{2}\) (000)  ones - Volunteer 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Sperm 3a	AP pool pool pool pool pool pool pool poo	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  epithelials  related to the condess below  1 minute and 15 seconds  epithelials  epitheli	Page	pool pool pool pool pool pool pool pool	POS	Yes Yes Yes No
3	Sperm 3e	AP PP P	time (s)  1 minute and 15 seconds 45 seconds 45 seconds 45 seconds ckend codes below  42 cla 42 cla 43 cla 44 cla 45 cla 46 cla 46 cla 47 cla 48 cla 49 cla 49 cla 40 cla	Page	Post	POS. 1-1 pos 1-2 pos 1	Yea Yea No
a) 4,500 coss - Volunteer 1 Th  ) Seman 6fr 1  2 1,700  5 1,700  5 1,700  1 1,700  1 1,700  1 1,700  1 1,700  1 1,700  1 1,700  1 1,700  2 1,700  2 1,700  2 1,700  3 1,700  4 1,700  5 1,700  5 1,700  6 1,700  6 1,700  6 1,700  7 1,700  7 1,700  8 1,700  8 1,700  9	Sperm  3e	AP PP P	time (s)  1 minute and 15 seconds 45 seconds 46 seconds 47 seconds 48 seconds 48 seconds 49 seconds 49 seconds 49 seconds 40 seconds	Page	Post	POS	Yea Yea No
1,000	Sperm 3a	AP pool pool pool neg	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  45 seconds  codes below	Page	Post	POS. 1-1 pos 1-2 pos 1	Yes Yes Yes No
1,000	Sperm 38 38 38 38 38 38 38 38 38 38 38 38 38	AP POPO POPO POPO POPO POPO POPO POPO P	time (s)  1 minute and 15 seconds  45 seconds  45 seconds  45 seconds  class below	Proceedings	Post	POS.	Yes Yes Yes No
a) \$\( \alpha \)	Sperm   3a   3a   3a   3a   3a   3a   3a   3	AP post post post post post post post post	time (s)  1 minute and 15 seconds 45 seconds 46 seconds 47 seconds 48 seconds 48 seconds 48 seconds 49 seconds 48 seconds 49 seconds 49 seconds 49 seconds 49 seconds 49 seconds 49 seconds 40 seconds	Page	Post	POS	Yes Yes Yes No

Proposed process - Volum	teer 3 - Thick fabric - Excision								
Sample ID Semen	dil'n Diff Slide barcode	Sperm 2+	AP pos	time (s) 2 minutes	p30 pos	Phadebas pos	l		No
	1/20	2+	pos	2 minutes	pos	pos	1	AP:	No
	1/50	de de	neg		pos pos	pos pos	l	17613-20190711_MAM P30:	No No No Yes No
	1/100	d+	neg		pos pos	pos pos	1	17860-23280702	No No
	These 4 samples were in 1/200 These samples are to be	a batch where ly	rsis buffer was ad	ded at the wrong stage.			l	new p30:	
	Second Batch - VDNAEXT201911	11-03	resempled using I	ne parcodes pelow.			l	18388-23290311	
	1/200	1+	neg		pos	pos	l		No
	1/200	1+	neg		pos pos	pos neg			No No No
	1/500	0	neg		pos	neg	l		No
- Volunte	er 3 - Thin fabric - Excision					8 (4HB 4T)			
Semen		whole sperm at room tempera	sperm heads	epithelials ekend	AP	time (s)	p30	Diff Slide barcode Sperm Phadebas	Yes
	4/20 and therefore are not sui	itable for analysis	s			50 seconds	-		No Yes
	4/50 All samples to be reprepa	ared and resamp	ied using the barr	todes below	906	1 minute	946 946		No Yes
	<del>1/100</del> 1/100				***	6-minute and 30 com 3 minutes	P44		Yes Yes
	1/200 1/200				neg		P44		Yes Yes
	/600	1			neg		pos		Yes
	1/20	0		d+	neg		pos	1+ pos	No
	1/20 1/50 1/50	0	<de< td=""><td>0</td><td>neg</td><td>1 minute and 50 secs</td><td>pos pos</td><td>1+ pos 1+ pos</td><td>No No</td></de<>	0	neg	1 minute and 50 secs	pos pos	1+ pos 1+ pos	No No
	1/50	0	<d+< td=""><td>1+</td><td>neg</td><td></td><td>pos</td><td>&lt;1+ pos</td><td>No No No</td></d+<>	1+	neg		pos	<1+ pos	No No No
	1/100	0	0	1+ <1+	neg		pos pos	1+ pos 1+ pos	No No
	1/200	0	0	<1÷	neg		neg	1 pos 1 pos	No No
	1/500	0	0	d+	neg		neg	<1+ pos	No No
	1/500	0	U	d+	neg		neg	10 (5xHB 5XT)	NO
ss - Volum Semen		Sperm	AP	time (s)	p30	Phadebas	ı	19377-20200109KA 18388-23290311	
	1/20	4+	neg		pos	pos	1		No
	1/50	2+ 1+	neg neg		pos pos	pos pos	1		No No
	1/50	1+	neg		pos pos	pos pos	l	17613-20190711 MAM	No No No No
	1/100	3+	neg		pos	pos	1	18388-23290311	No
	1/200	10	neg neg		pos pos	pos pos	l		No No
	1/500 1/500	d+	neg		neg pos	neg pos	l		No No
						9 (4xHB 5xT)			_
- Donor 4 Semen	diffe	whole sperm		epithelials	AP	time (s)	р30		
	1/20	0 <1+	<1+	1+	neg		pos pos	1	Yes Yes
	1/50	0	<1+	<1+ 1+	neg		neg	1+ pos	Yes Yes
	1/100	0	0	1+	neg		pos pos	1+ pos	Yes
	1/200	0	0	1+	neg		neg neg		Yes Yes
	1/200	0	0	1+	neg		neg	<1+ pos	Yes
	1/500 1/500	0	0	d+	neg		neg neg	0 pos <1+ pos	Yes Yes
								10 (5xHB 5XT)	
ss - Donor	4-C								
is - Donor Semen	4-C	Sperm	AP	time (s)	p30		ı		Ver
s - Donor Semen	1/20 1/20	1+ 3+	neg neg	time (s)	pos pos	pos pos		17613-20190711_MAM	Yes Yes
is - Donor Semes	di'n 1/20 1/20 1/50	1+ 3+ <1+	neg neg	time (s)	pos pos pos	pos pos pos	Slide hard	17613-20190711_MAM I to read because of bia: 17860-23280702	Yes
Semei	diri 1/20 1/36 1/50 1/50 1/50 1/100	1+ 3+ <1+ <1+ 1+	neg neg neg neg	time (s)	pos pos pos pos	pos pos pos pos	Slide hard	17613-20190711_MAM I to read because of bin 17869-23280702	Yes Yes Yes
Semer	1/20 1/20 1/50 1/50	1+ 3+ <1+	neg neg neg	time (s)	pos pos pos	pos pos pos	Slide hard	17613-20190711_MAM I to read because of bias 17866-23280702	Yes Yes Yes Yes Yes
Semes	dirk 1/20 1/20 1/50 1/50 1/100 1/100 1/200	19 30 40 40 19 19 40 40	gen gen gen gen gen gen	time (s)	pos pos pos pos pos pos	905 905 905 905 905 905 905	Slide hard	17613-20190711_MAM I to read because of bia 17800-23240702	Yes Yes Yes Yes
Semen	dire 1/20 1/25 1/50 1/50 1/100 1/100 1/200	10 30 410 410 10 10 410	gen gen gen gen gen gen	time (s)	pos pos pos pos pos pos pos pos neg neg	905 905 905 905 905 905 906 906 906	Slide hard	17613-20190711_MAM to read because of bin 17860-23280702	Yes Yes Yes Yes Yes Yes
Semen	difficient of the state of the	19 39 40 40 19 19 40 40 40 40 40 40 40 40 40 40 40 40 40	neg neg neg neg neg neg neg neg		pos pos pos pos pos pos neg neg	pos		to read because of bis 17860-23280702	Yes Yes Yes Yes Yes Yes Yes
Semes	diffn 1/2c 1/2c 1/2c 1/2c 1/2c 1/2c 1/2c 1/2c	19 39 419 419 19 419 419 19	neg neg neg neg neg neg neg neg	time (s)  epitbuluki 5	pos pos pos pos pos pos pos pos neg neg	905 905 905 905 905 905 906 906 906	р30	to read because of bile 17860-23280702	Yes Yes Yes Yes Yes Yes Yes
Semen	diffication of the state of the	1+ 3+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+	neg	epithelials	pos	pos	p30 pos pos	to read because of bin 17860-23280702  Diff Side baroods Sparm Photobin 34 pm 33 pm	Yes
- Volunte - Jemei	diffication of the second of t	19 38 419 419 19 419 419 419 419 419 419 419	neg	epithelisis	pos pos pos pos pos pos pos neg neg neg	pos	p30 pos pos neg	10 read because of bias 17860-23280702	Yes
- Volunte Semen	diffication of the second of t	1+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+ 3+	neg   neg		pos	pos	p30 pos pos neg	Deff Side barcode   Sparm   Pheddeat   31   50   50	Yes
- Volument	HETT WAR	18 38 41 41 41 41 41 41 41 41 41 41 41 41 41	neg	epithelius 1s - cts 0 0 0	pos pos pos pos pos pos neg neg neg neg neg neg	pos   to (SeHB SXT)	p30 pos pos neg pos neg neg	10 read because of bia 17860-23280702	Yes
- Volunte - Jemei	diffication of the control of the co	14	neg	######################################	pos pos pos pos pos neg neg neg neg neg neg neg neg	pos   to (SeHB SXT)	p30 pos pos neg neg neg neg	Degrade hercose of bias 17860-23280702	Yes
- Voluntes	differ  Most   10 10 10 10 10 10 10 10 10 10 10 10 10 1	neg   neg	epithelisis 	pos pos pos pos pos pos neg neg neg neg neg neg neg	pos   to (SeHB SXT)	p30 pos pos neg pos neg neg	Deff Side barcode   Sperm   Phatidute	Yes	
- Volunte Semen	diffication of the control of the co	14	neg   neg	epithalistic    14	pos pos pos pos pos pos neg neg neg neg neg neg neg	900   900	p30 pos pos neg neg neg neg	Def Side barcode	Yes
- Volunte Semen	diffication of the control of the co	18 38 44 18 18 48 48 48 48 48 48 48 48 48 48 48 48 48	neg   neg	######################################	pos pos pos pos pos pos neg neg neg neg neg neg neg neg	900   900	p30 pos pos neg neg neg neg	Def Side barcode	Yes
- Volunte Semen	diffication of the state of the	14 38 44 44 44 44 44 44 44 44 44 44 44 44 44	neg   neg	epithalistic    14	pos pos pos pos pos pos neg neg neg neg neg	post	p30 pos pos neg neg neg neg	Def Side barcode	Yes
- Volunte Semei	diffication of the second of t	18 18 18 18 18 18 18 18 18 18 18 18 18 1	neg   neg	epithalistic    14	pos	post	p30 pos pos neg neg neg neg	Def Side barcode	Yes
- Voluntie Semei	diffication of the control of the co	18		epithalistic    14	pos	post	p30 pos pos pos neg neg neg neg	Deff Side barcode   Sperm   Phatidute	Yes
- Volunts Semen	difference of the control of the con	11 12 12 12 12 12 12 12 12 12 12 12 12 1		epithalistic    14	pos	post	p30 pos pos pos neg neg neg neg	Def Side barcode	Yes
- Volumes Senter  se - Volume Senter	##Th ##Th ##Th ##Th ##Th ##Th ##Th ##Th	14 34 34 34 34 34 34 34 34 34 34 34 34 34	neg   neg	epithalistic    14	pos    post	p30 pos pos neg pos neg pos neg	Deff Side barcode   Sperm   Phatidute	Yes	
- Volument Semei	###  #	11 12 12 12 12 12 12 12 12 12 12 12 12 1	neg   neg	epithalistic    14	pos    Post   Phart	p30 pos pos neg pos neg pos neg	Deff Side barcode   Sperm   Phatidute	Yes  Yes  Yes  Yes  Yes  Yes  Yes  Yes	
- Volumeter sa - Volumeter - Volumeter - Volumeter - Volumeter	### ### ### ### ### ### ### ### ### ##	18 38 41 41 41 41 41 41 41 41 41 41 41 41 41	neg   neg	epitheliudi 12 - 12 - 12 - 12 - 12 - 12 - 12 - 12 -	pos	post	p300 pos	Diff Side baroods Sparm Photebas 29 pm 24 pm 29 pm 29 pm 29 pm 20	Yes
- Volumeter sa - Volumeter - Volumeter - Volumeter - Volumeter	### ### ### ### ### ### ### ### ### ##	18 38 41 41 41 41 41 41 41 41 41 41 41 41 41	Reg   Reg	epitheliulis	POS    pos	p300 pos	10 read because of blat 17860-23280702	Yes	
- Volumeter sa - Volumeter - Volumeter - Volumeter - Volumeter	### ### ### ### ### ### ### ### ### ##	18		### ##################################	pos	Post	p300 pos	Def Side barcode	Yes
- Volunte seme	different control of the control of	11 12 12 12 12 12 12 12 12 12 12 12 12 1		### ##################################	POD    Post	p300 pos	Def Side barcode   Sparm   Phedebas   Sparm   S	Yes	
- Volunte seme	different differ	11   12   13   14   15   15   15   15   15   15   15	neg   neg	epithelisis	POS	Post	p300 pool pool pool pool pool pool pool po	Diff Side harcode	Yes
- Volunts Semei ss - Volunts Semei	### ### ### ### ### ### ### ### ### ##	18		epitheliulis  1	Dec    Post	p300pos	Deff Side barcode   Sperm   Phadebas   39   968   29   968   29   968   29   968   29   968   29   968   20	Yes	
Semes  - Volunts Semes  - Volunts Semes  - Volunts Semes	different differ	11   12   13   14   15   15   15   15   15   15   15		epithelisis	POS	Post	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes
- Volunts	### ### ### ### ### ### ### ### ### ##	11 12 12 12 12 12 12 12 12 12 12 12 12 1		### ##################################	POS	post	p300 pool pool pool pool pool pool pool po	Def Side harcode   Sparm   Phedeba   3	Yes
Semes  - Volunte Semes  - Volunte Semes  - Volunte Semes	### ### ### ### ### ### ### ### ### ##	18	Reg   Reg	egitheliah	POINT   POIN	post	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes
- Volunts	### ### ### ### ### ### ### ### ### ##	11 12 12 12 12 12 12 12 12 12 12 12 12 1	Reg   Reg	### ##################################	POINT   POIN	position	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes
- Volunts Semes  ss - Volunts Semes  - Volunts Semes  - Volunts Semes	### ### ### ### ### ### ### ### ### ##	11		### ##################################	POD    post	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes	
- Volunte Semes  s= Volunt Semes  - Volunte Semes  - Volunte Semes	###  #	14   14   14   15   15   15   15   15	neg   neg	### ##################################	POD	DOS   DOS   DOS	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes
- Volument semen s= Volument Semen - Volument Semen	###  #	14   34   34   34   34   34   34   34	neg   neg	### ##################################	POD    DOS   DOS   DOS	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes	
- Volunts Semei  s- Volunts Semei  - Volunts Semei	### ### ### ### ### ### ### ### ### ##	14	neg   neg	### ##################################	POID	post	p300 pool pool pool pool pool pool pool po	Deff Side barcode   Sperm   Phediches	Yes
- Volunts Semei  s- Volunts Semei  - Volunts Semei	### ### ### ### ### ### ### ### ### ##	14   14   14   14   14   14   14   14	neg   neg	### ##################################	POD    post	p30 pos pos pos pos pos pos pos pos pos pos	Deff Side barcode   Sperm   Phediches	Yes	

ficroscopy Results:	•
apelift - D4 thick: Proposed process appears better than the curr	rent process and consistent with the diff slide process. No different between heatblock and thermomixer
apelift - D4 thin: Proposed process appears better than the curre	ent process and consistent with the diff slide process. Heatblock better
apelift - V1 thick: Proposed process appears better than the curr	ent process and worse than the diff slide process. Heatblock better
apelift - V1 thin: Proposed process appears better than the curre	mt process and worse than the diff slide process. Heatblock better
apelift - V3 thick: Proposed process appears consistent with the	current process and worse than the diff slide process. No difference between heatblock and thermomixer.
apelift - V3 thin: Proposed process appears better than the curre	ent process and consistent with the diff slide process. Heatblock better
xcision - D4 thick: Proposed process appears better than the cur	rent process and better than the diff slide process. Heatblock better
xcision - D4 thin: Proposed process appears better than the curr	ent process and worse than the diff slide process. No difference between heatblock and thermomixer
xcision - V1 thick: Proposed process appears better than the cur	rent process and worse than the diff slide process. No difference between heatblock and thermomixer
xcision - V1 thin: Proposed process appears better than the curr	ent process and better than the diff slide process. No difference between heatblock and thermomixer
xcision - V3 thick: Proposed process appears better than the cur	rent process and worse than the diff slide process. No difference between heatblock and thermomixer.
xcision - V3 thin: Proposed process appears better than the curr	ent process and better than the diff slide process. Heatblock better.
otton swabs - D4: Proposed process appears better than the cur	rent process and better than the diff slide process. No difference between heatblock and thermomixer.
otton swabs - V1: Proposed process appears better than the cur	rent process and worse than the diff slide process. No difference between heatblock and thermomixer.
otton swabs - V3: Proposed process appears better than the cur	rent process and better than the diff slide process. Heatblock better
	ı
roposed process better than current process	14
roposed process consistent with current process	1
roposed process worse than current process	0
roposed process better than diff slide	5
roposed process consistent with diff slide	3
roposed process worse than diff slide	7
	1 -
hermomixer better:	0
leatblock better:	7
io difference:	8
hadehas results:	
urrent process better:	

Current process better: Proposed process better No difference:

Positive thermomixer: Positive heatblock:

	Current										Proposed									
	Swabs		Tape lift - Thick fabric		Tape lift - Thin fabric		Excision - Thick Fabric		Excision - Thin fabric		Swabs		Tape lift - Thick fabric		Tape lift - Thin fabric		Excision - Thick fabric		Excision - Thin fabric	
	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit
Donor 4	1/20	1/100	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/200	1/200	1/100	1/200	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/500	1/500
Volunteer 1	1/20	1/50	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/200	1/200	1/50	1/50	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/500	1/500
Volunteer 3	1/50	1/200	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/100	1/100	1/50	1/50	p30 neg	p30 neg	p30 neg	p30 neg	1/500	1/500	1/200	1/500

results:

		Current									Proposed									
	Swabs		Swabs Tape lift - Thick fabric		Tape II	ft - Thin fabric	Excision	- Thick Fabric	Excisio	n - Thin fabric		Swabs	Tape III	ft - Thick fabric	Tape II	ft - Thin fabric	Excision	n - Thick fabric	Excision	n - Thin fabric
	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit	sensitivity	detection limit
Donor 4	AP neg	AP neg	AP neg	AP neg	AP neg	AP neg	1/20	1/20	1/20	1/20	AP neg	AP neg	AP neg	AP neg	AP neg	AP neg	1/20	1/50	AP neg	AP neg
Volunteer 1	NE	1/20	AP neg	AP neg	AP neg	AP neg	1/50	1/50	NE	1/20	AP neg	AP neg	AP neg	AP neg	AP neg	AP neg	1/20	1/20	AP neg	AP neg
Volunteer 3	NE	1/20	AP neg	AP neg	AP neg	AP neg	1/50	1/50	NE	1/50	AP neg	AP neg	AP neg	AP neg	AP neg	AP neg	1/20	1/20	AP neg	AP neg

Experiment 4 part 3 cont - 299 samples Experiemet 4 part 3 - 200 samples Experiment 4 amended - 20 samples Total : 519 samples

Lab No.	External Id	Specimen Description	tails	heads	epis	AP	p30	Diff batch	quant	diff heads	profile Comment
-	-S	Pos control test sample 3	0	1+	<1+	90s	pos		0.038	2+	Clear maj Sperm prof, small amoun pos control lot:
-	-S	Pos control test sample 2	<1+	2+	0	100s	pos		0.0421	2+	Clear maj Sperm prof, small amoun pos control lot:
-	-S	Pos control test sample 1	0	1+	<1+	80s	pos		0.094	3+	Clear maj Sperm prof, small amoun pos control lot:

mber Ur Number	FBQUAN XPB22	Ext ID	Lab Number	Ur Number	FBQUAN XPB22	Ext ID	La	b Numbe Ur Number	FBQUAN XPB22	Ext II
	2.8152 MIX OK				0.0618 MIX OK				2.8152 MIX OK	
	0.0618 MIX OK				0.0235 OK MIX				2.3788 OK MIX	
	2.3788 OK MIX				0.0615 OK MIX				4.0989 OK	
	0.0235 OK MIX				0.0509 MIXT OK				3.4103 OK	
	4.0989 OK				0.0518 MIX OK				3.2669 MIX OK	
	0.0615 OK MIX								1	
	3.4103 OK				0.062 MIX OK				3.6555 MIX OK	
	0.0509 MIXT OK				0.0954 MIX OK				3.0061 MIX OK	
	3.2669 MIX OK				0.0218 OK EXT PK>				0.8756 MIX OK	
	0.0518 MIX OK				0.0549 OK MIX				1.8872 OK MIX	
					0.0733 MIXT OK				2.6422 MIXT OK	
					0.0565 MIX OK				4.4017 OK	
	3.6555 MIX OK				0.0795 MIX OK				2.6437 MIX OK	
	0.062 MIX OK				0.1422 OK MIX				3.7061 OK	
	3.0061 MIX OK				0.0526 MIX OK				3.4491 MIX OK	
	0.0954 MIX OK				0.1038 OK				2.5724 MIXT OK	
	0.8756 MIX OK				0.0423 MIXT OK				4.0621 MIXT OK	
	0.0218 OK EXT PK>				0.0945 OK MIX				2.4179 OK MIX	
	1.8872 OK MIX				0.048 OK MIX				2.0725 OK MIX	
	0.0549 OK MIX				0.1064 OK MIX				2.3894 OK MIX	
	2.6422 MIXT OK				0.0586 MIX OK				2.4478 OK	
	0.0733 MIXT OK				0.0300 WIIA OK				2.4470 OK	
	4.4017 OK			Average	0.06707			Average	2.90997	
	0.0565 MIX OK			Max	0.1422			Max	4.4017	
	2.6437 MIX OK			Min	0.0218			Min	0.8756	
	0.0795 MIX OK			IVIIII	0.0216			IVIIII	0.8730	
	3.7061 OK									
	0.1422 OK MIX			From initial	slide analysis spreadsheet					
	3.4491 MIX OK			Dlys micro		Orig micro	Auguant			
	0.0526 MIX OK			DIYS ITIICIO	Av quant 0 0.00885	Orig micro	Av quant 0 0.313073			
	2.5724 MIXT OK			<1+	0.0756	-1.				
						<1+	0.381583			
	0.1038 OK			1+	0.09833	1+	0.60497			
	4.0621 MIXT OK			2+	0.60653	2+	1.33675			
	0.0423 MIXT OK			3+	0.61955	3+	1.87654			
	2.4179 OK MIX			4+	2.61989	4+	20.754			
	0.0945 OK MIX									
	2.0725 OK MIX									
	0.048 OK MIX									
	2.3894 OK MIX									
	0.1064 OK MIX									
	2.4478 OK									
	0.0586 MIX OK									

Selection of three previously made pos D Lys pos controls

Lab no tails heads epi's AP p30 Diff Lysis batch ID

DL sp micro Slys Quant (1st run)

Client re	ference	tails	heads	AP	p30	Diff Lysis batch ID	diff lysis micro result	Slys Quant (1st run)	Other notes	tails > heads
		0	<1+			CWDMAX20140102_02	3+		casefile FB2CFB93	
		0	<1+	n/a	n/a	CWDMAX20140102_02	0	0.0172		
		0	<1+			CWDMAX20140102_02	0	0		
		0	<1+	n/a	n/a	CWDMAX20140107_02	0	0.0147	exam notes FBCM24	
		0	<1+			CWDMAX20140123_01	0	0.0231	casefile FB2CFB95	
		0	1+			CWDMAX20140128_01	1+		casefile FB2CFB93	
		0	1+			CWDMAX20140128_01	2+		exam notes FBP34	
		0	2+	n/a		CWDMAX20140128_01	3+	1.45		
		0	3+			CWDMAX20140128_01	2+	5		
		0	2+			CWDMAX20140204_03	4+		casefile FB2CFB93	
		0	2+	n/a		CWDMAX20140204_03	1+	0.265	caserne i bzer bss	
		0	0			CWDMAX20140204_03	0		exam notes FBP35	
		0	0			CWDMAX20140204_03	0	0	CAUTI HOLES I DI 33	
		<1+	2+	n/a		CWDMAX20140210_01	2+	0.0654		
		0	1+			CWDMAX20140210_01	1+	0.327		
		0	1+			CWDMAX20140210_01	2+	0.706		
		0	2+				4+	0.945		
		0				CWDMAX20140210_01 CWDMAX20140210_01	2+			
			<1+	٠.	-	_		0.0155		
		0	1+ <1+	n/a		CWDMAX20140210_01	2+ 1+	0.262		
						CWDMAX20140210_01		0.00278		
		1+	3+			CWDMAX20140210_01	3+	1.45		
		0	1+			CWDMAX20140210_01	1+	0.175	casofila ED2CEDO7	
		0	<1+			CWDMAX20140227_01	4+		casefile FB2CFB97	
		0	<1+			CWDMAX20140227_01	3+	0.344		
		0	1+	n/a		CWDMAX20140227_01	3+	0.259		
		0	1+			CWDMAX20140227_01	3+	0.177	cacofila ED2CEDO7	
		0	0			CWDMAX20140307_01	0		casefile FB2CFB97	
		0	<1+			CWDMAX20140307_01	1+		exam notes FBP35	
		0	1+			CWDMAX20140318_01	4+	0.338		
		0	1+ 2+			CWDMAX20140318_01 CWDMAX20140318_01	3+ 3+	0.229 0.173		
		0	0			CWDMAX20140318_01 CWDMAX20160105_01	0	0.173		_
		0				_	0			
			<1+			CWDMAX20160127_02		0.0007		
		0	<1+ <1+			CWDMAX20160127_02 CWDMAX20160202_01	0	0.0008 0.0007		
		0	<1+ 0			CWDMAX20160202_01 CWDMAX20160202_01	0 4+	0.0007		
		0				_	4+			
		0	<1+ 0			CWDMAX20160202_01 CWDMAX20160209_01	4+	2.1598 0.4081		
		0	1+			CWDMAX20160209_01	3+	0.4081		
		0	1+			CWDMAX20160209_01	3+ 4+	3.9821		
		0				_				
		0	3+ 1+	n/a		CWDMAX20160217_01	3+ 1+	0.3902 0.1257		
		0	1+ <1+			CWDMAX20160217_01 CWDMAX20160225_01	1+ 4+	0.1257 0.756		
		0	<1+			CWDMAX20160225_01	4+	2.3197		
		<1+	2+			CWDMAX20160225_01	4+	3.7443		
		0	2+ <1+			CWDMAX20160225_01	4+	3.7443		
		0	0	n/a		CWDMAX20160225_01	3+	1.7075		
		0	1+			CWDMAX20160225_01	3+ 4+	1.8191		
		0	1+			CWDMAX20160225_01	3+	0.4547		
		0	1+				3+	0.4547		
		0				CWDMAX20160225_01	3+			
		0	<1+			CWDMAX20160225_01		0.1796		
		0	1+ 0	n/a n/a		CWDMAX20160225_01 CWDMAX20160225_01	4+ 2+	0.7121 0.3429		
		0	<1+			CWDMAX20160225_01	1+	0.3429		
		0				_				
		0	<1+ 0			CWDMAX20160225_01	2+ 3+	0.1666 0.0647		
		0	2+			CWDMAX20160225_01 CWDMAX20160229_01	2+	0.0647		
		1+	2+			CWDMAX20160229_01	3+	0.9548		
		0	1+			CWDMAX20160229_01	2+	0.9066		
		0				CWDMAX20160229_01	2+ 4+	1.093		
		0	1+ 1+			_	4+	1.3892		
		<1+	1+ <1+			CWDMAX20160229_01 CWDMAX20160229_01	4+ <1+	0.0065		
		0	0			CWDMAX20160229_01	4+	0.162		
		0	0			CWDMAX20160229_01	2+	0.3211		
		0	<1+			CWDMAX20160229_01	3+	0.2558		
		0	2+			CWDMAX20160229_01	4+	4.0784		
		0	3+			CWDMAX20160229_01	3+	2.1695		
		1+	4+			CWDMAX20160229_01	4+	20.754		
		0	<1+			CWDMAX20150114_01	1+	0.0631		
		0	1+			CWDMAX20150114_01	2+	0.0031		
		0	<1+			CWDMAX20150114_01	1+	0.0454		
		0	1+			CWDMAX20150114_01	2+		casefile FB2CFB112	
		0	3+			CWDMAX20150114_01	3+	0.373		
		0	1+			CWDMAX20150114_01	2+	0.126		
		<1+	<1+			CWDMAX20150114_01	1+	0.00614		
		0	<1+			CWDMAX20150121_01	1+		casefile FB2CFB114	
		0	<1+			CWDMAX20150121_01	2+	0.0314		
		0	1+			CWDMAX20150121_01	<1+	0.0853		
		0	<1+	n/a	n/a	CWDMAX20150121_01	<1+	0.135		

CWDMAX20150203\_01 CWDMAX20150212\_01 CWDMAX20150223\_01 CWDMAX20150305\_02 CWDMAX20150310\_01 CWDMAX20150310\_01 CWDMAX20150331\_01

CWDMAX20160310\_01
CWDMAX20160314\_01
CWDMAX20160317\_01
CWDMAX20160322\_01
CWDMAX20160324\_02
CWDMAX20160401\_01
CWDMAX20160412\_02
CWDMAX20160420\_01
CWDMAX20160426\_01

Orig micro (heads)	D Lys micro Count	t av quant	No instance	e where more speri	m with tails was > s	sperm heads on	original micro	Orig micro (heads)	D Lys micro	Coun
0	0	4 0.01005	In all instan	nces where 1+ tails	were seen, 2+ or g	reater heads wh	iere seen	0	0	4
0	<1+	0 N/A	No instance	es of 2+ or more sp	erm with tails seer	1		<1+	0	7
0	1+	0 N/A						1+	0	0
0	2+	2 0.332	Number of	instances Orig mici	ro > D lys micro		10	2+	0	0
0	3+	2 0.8861	Number of	instances Orig mici	ro = D lys micro		17	3+	0	0
0	4+	3 0.322467	Number of	instances Orig mici	ro < D lys micro		52	4+	0	0
<1+	0	7 0.008171						0	<1+	0
<1+	<1+	2 0.07075	Number of	times D lys micro 4	1+	19		<1+	<1+	2
<1+	1+	7 0.039717	Number of	times D lys micro 3	3+	19		1+	<1+	1
<1+	2+	3 0.071167	Number of	times D lys micro 2	2+	15		2+	<1+	0
<1+	3+	4 0.20595	Number of	times D lys micro 1	L+	12		3+	<1+	0
<1+	4+	5 1.83406	Number of	times D lys micro <	:1+	3		4+	<1+	0
1+	0	0 N/A	Number of	times D lys micro 0	)	11		0	1+	0
1+	<1+	1 0.0853						<1+	1+	7
1+	1+	4 0.159245	Dlys micro	Av quant	Orig micro	Av quant		1+	1+	4
1+	2+	7 0.314329	0	0.008855	0	0.313073		2+	1+	1
1+	3+	6 0.3772	<1+	0.0756	<1+	0.381583		3+	1+	0
1+	4+	6 1.555583	1+	0.098333	1+	0.60497		4+	1+	0
2+	0	0 N/A	2+	0.606533	2+	1.33675		0	2+	2
2+	<1+	0 N/A	3+	0.619553	3+	1.87654		<1+	2+	3
2+	1+	1 0.265	4+	2.619889	4+	20.754		1+	2+	7
2+	2+	2 0.5101						2+	2+	2
2+	3+	3 0.8432	Number of	instances orig micr	ro pos, diff micro n	eg	7	3+	2+	1
2+	4+	4 2.388175	all instance	s of orig micro pos	, diff neg had <1+ i	n orig micro		4+	2+	0
3+	0	0 N/A						0	3+	2
3+	<1+	0 N/A	Number of	instances orig micr	ro neg, diff micro p	ios	7	<1+	3+	4
3+	1+	0 N/A		f orig micro neg, di			2	1+	3+	6
3+	2+	1 5		f orig micro neg, di	•		2	2+	3+	3
3+	3+	4 1.095675	instances o	f orig micro neg, di	iff pos 4+		3	3+	3+	4
3+	4+	0 N/A						4+	3+	0
4+	0	0 N/A						0	4+	3
4+	<1+	0 N/A						<1+	4+	5
4+	1+	0 N/A						1+	4+	6
4+	2+	0 N/A	2014	4 31				2+	4+	4
4+	3+	0 N/A	201					3+	4+	0
4+	4+	1 20.754	201					4+	4+	1
	total	79	total	79						

Lab No.	Id External Id Received Specimen	Specimen Descrip	Relation Tests Status	Lab No. Id Ex	xternal Id Received Specimen	Specimen Descrip	Relation Tests Status	barcode batch ID
	2-Feb-17 EFRAC Swab		XPLEXM		·	1 in 20 diln #2 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 20 dil #2S	XPLEXM			1 in 20 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 20 dil #3E	XPLEXM			1 in 20 diln #3 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 20 dil #3S	XPLEXM		-Feb-17 VALS	1 in 20 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 20 dil #4E	XPLEXM		-Feb-17 VALE	1 in 20 diln #4 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 20 dil #4S	XPLEXM		-Feb-17 VALS	1 in 20 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 50 dil #1E	XPLEXM		-Feb-17 VALE	1 in 50 diln #1 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 50 dil #1S	XPLEXM		-Feb-17 VALS	1 in 50 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 50 dil #2E	XPLEXM		-Feb-17 VALE	1 in 50 diln #2 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 50 dil #2S	XPLEXM		-Feb-17 VALS	1 in 50 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 50 dil #3E	XPLEXM		-Feb-17 VALE	1 in 50 diln #3 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 50 dil #3S	XPLEXM		-Feb-17 VALS	1 in 50 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 50 dil #4E	XPLEXM		-Feb-17 VALE	1 in 50 diln #4 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 50 dil #4S	XPLEXM		-Feb-17 VALS	1 in 50 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 100 dil #1E	XPLEXM		-Feb-17 VALE	1 in 100 diln #1 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 100 dil #1S	XPLEXM		-Feb-17 VALS	1 in 100 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 100 dil #2E	XPLEXM		-Feb-17 VALE	1 in 100 diln #2 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 100 dil #2S	XPLEXM			1 in 100 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 100 diln #3 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 100 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 5 diln #1 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 5 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 100 diln #4 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 100 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 200 diln #1 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 200 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 200 diln #2 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 200 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 200 diln #3 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 200 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab 2-Feb-17 SFRAC Swab		XPLEXM XPLEXM			1 in 200 diln #4 (epi 1 in 200 diln #4 (sp)	XPLEXM XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 500 diln #1 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 500 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 500 diln #2 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 500 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 500 diln #3 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 500 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 500 diln #4 (epi	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 500 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 5 diln #2 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 5 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 5 diln #3 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 5 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 5 dil #4E	XPLEXM			1 in 5 diln #4 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 5 dil #4S	XPLEXM			1 in 5 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 10 dil #1E	XPLEXM		-Feb-17 VALE	1 in 10 diln #1 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 10 dil #1S	XPLEXM			1 in 10 diln #1 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 10 dil #2E	XPLEXM			1 in 10 diln #2 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 10 dil #2S	XPLEXM			1 in 10 diln #2 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab	1 in 10 dil #3E	XPLEXM			1 in 10 diln #3 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 10 diln #3 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 10 diln #4 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab		XPLEXM			1 in 10 diln #4 (sp)	XPLEXM	
	2-Feb-17 EFRAC Swab		XPLEXM			1 in 20 diln #1 (epi)	XPLEXM	
	2-Feb-17 SFRAC Swab	1 in 20 dil #1S	XPLEXM		-Feb-17 VALS	1 in 20 diln #1 (sp)	XPLEXM	

#### Feedback

The proposed experiments will use swabs. Is any of the data that you quote (where you have looked quantitatively at the old samples' exam slides vs their diff slides) from swabs or was it all scrapings/cuttings? I would be curious to know whether the quantitative variation we are seeing could be related to the substrate/sampling method.

I don't understand where the AP and p30 fit into testing the reliability of microscopy. You know there's sperm on the swabs because you put it there. For a whole host of reasons AP/p30 are poorly correlated with spermatozoa concentrations.

I don't think the presumptive testing component is necessary – we know that any number of As above, the idea was to include it as the testing previously has been done in isolation. In combinations of results between AP/p30/micro could have legitimate explanations, so I'm not addition, work on p30 and AP (from validation documentation located) has been performed sure what this testing will show.

Has there been a background lit search performed?

- 1.1 Background results from earlier investigation should really be written up in a separate section as a pilot study
- 1.1 Background paragraph 3 should be in the Purpose and scope section as it is the aim of the study.
- 5.1 section ii this needs to be clearer. A short table consisting of a brief outline of each batches contents would be ideal.
- 6. Results and Data Compilation How will any statistical differences be determined? You have repeated samples measured with a difficult categorical system (0-4; where the difference between 0 and 1 is not the same as the difference between 3 and 4) in a probably non-normal population across pre and post treatment groups. This is not easy to analyse statistically if at all, in its current form.

Other minor wording options provided

#### Response

The initial data mining was done on a mix of substrates as it was taken from samples that had been processed through diff lysis extraction. It was considered at the time to record the sample type. There have been a number of discussions and this has included substrate type, however there are so many variables to consider such as sample type, amount of sample, sample source, presence / absence of other biological material & other substances such as lubricant, investigation of operator variability (both at examination and the process of making the slides at extraction) etc. This initial investigation needed to be more focussed, and substrate (as well as other elements will be something that may well come into the investigation further on.

The idea of recording AP / p30 was to include some data for education purposes on how sperm micro sensitivity and p30 & AP testing sensitivity relate – we know that the relative levels of sperm, p30 and AP will vary between individuals, and we don't do quantitative testing. There are also variables such as sampling method, whether an item has been washed, time since deposition etc. Agree that definitely no absolutes can be drawn from any of our data. However, the thought was that the lab had never (from records that were located) tested all of the elements together – presumptive test, micro and extraction results. In the past validations were all performed on individual steps in isolation (p30 or extraction etc.). A consideration was to include testing from a range of donors, but as with substrates above, there are too many variables which would expand the size and number of experiments, so it was decided would start small, and as more information is gathered start to test some of the variables.

As above, the idea was to include it as the testing previously has been done in isolation. In addition, work on p30 and AP (from validation documentation located) has been performed on dilutions of the material eluted / suspended from neat semen placed on a substrate (e.g. neat semen added to a substrate, water added, then dilutions of that water tested), whereas in this project we propose to test diluting the semen prior to addition to the substrate, this should mimic casework closer.

A search was conducted to find work performed on comparisons of microscopy results vs profiling sensitivity, microscopy results vs p30 or AP testing, however no papers where processing was performed the same or similar to the techniques we employ could be located. The individual elements (e.g. AP testing, ABA card p30 test, H & E stain etc.) are well documented, but could not locate documentation of anyone performing a process the same or very similar to ours where a suspension is made right from the start.

Will write it as a sub-section

Agree, document to be updated

Agree, document to be updated

There was no intention to conduct statistical analysis for the reason noted, however data trends will be looked at and considered. A lack of trend may itself also indicate where an issue may lie (e.g. may lead to reproducibility / repeatability investigations as a next step)

mostly adopted and changed

#### Options put to management team

Feedback

Option A: Attempt to improve the method for making a suspension in Evidence recovery to try to release more sperm from the substrate Option B: Submit all samples straight for Diff Lysis and triage processing after reviewing the slide Something else (with suggestion)

4 votes

3 votes

can we look at a variation of Option B to include a soak, spin and retain for Ap/p30 before submission

Sure that is an option - it's just not a workflow Emma or to Analytical and then to search for these depending on sperm findings/or lack thereof? Might have already been accounted for but am questioning the loss of AP/p30 point

myself discussed / thought of

If a sample was to go straight to diff could you not do AP/p30 on the supernatant as done for phadebas. Or you could do the AP by wetting the swab and spotting onto filter paper to AP test prior Similar to suggestion above to submitting then do p30 to supernatant post extraction

I know this will be challenging, but I would think a risk assessment (in terms of result outcomes), and cost analysis of the Option A and Option B would help in guiding the decision

Option B clearly optimised profile outcomes – however comes at a time/resources cost that may or may not be sustainable. Would also need to consider PSA result as I would think that still has some

RT1 and RT2 had a discussion on both options and we have all voted on Option A

This paper describes the sperm elution method developed at Cellmark: https://www.clinicalkey.com.au/#!/content/playContent/1-s2.0-

\$1355030612000676?returnurl=https:%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F\$135503 a water elution for presumptive testing, and then looking at 0612000676%3 F show all %3 D true & referrer = https: %2 F%2 F www.ncbi.nlm.nih.gov

Also found this article about using resuspension of swabs to improve recovery of cells (not sperm specifically)

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0116351

My initial thought is option A. While I see benefit with Option B, we would lose the ability to detect intact sperm. If a risk assessment was undertaken, and it was deemed this to be an acceptable risk, then we could look further into options surrounding B.

Slide elution method

see suggestions above for possibly continuing with AP/p30

Nil required

Response

Hulme et al Sperm elution: An improved two phase recovery method for sexual assault samples:

Method in broad strokes appears to be a bit similar to taking the diff slide after the initial separation in the diff lysis

Adamowicz et al Evaluation of Methods to Improve the Extraction and Recovery of DNA from Cotton Swabs for Forensic Analysis: Very fiddly, somewhat impractical extraction pre-lysis method

Spiker et al found that cutting a piece of the swab (approx 1/4) then plaing onto the slide, adding 45uL water and then tapping the piece of swab with a stick for 10sec was the best method, compared against a swirling method and a "tube elution" method which appeared to be similar to the spin basket method trialled as part 2 of this project. Tube elution performed worse compared to the direct slide methods

#### Pros and cons put to management team

Option A		Option B	
Pro	Con	Pro	Con
More efficient processing downstream from ER	May slow down ER processing	Not losing sperm during screening = maximise profile recovery	Losing capability to do AP / p30
May eliminate need to make a	Further method development required (may involve reassessing / verifying new AP &		Doesn't help analytical with diff
diff slide	p30 protocols)	Could implement straight away	lysis extraction numbers
maintain AP/p30 capacity  More cost effective		Speeds up initial processing in ER not doubling up on microscopy	Requires some FR / workflow development
		Could use Y-quant to assist in workflow choice	

#### Feedback

I notice in 5.2 the MISC and SUPNAT to be stored frozen. I assume these will then be thawed at some stage in order to test – should this period of freezing be noted in 5.2 eg. stored frozen week prior to processing to replicate usual maximum routine processing time-frames" for x days to mimic process of sperm searching during DLYS step (and requirement to screen for fluid after that) or words to that effect....

Minor text change: Section 5.5: All DNA extracts will "be" processed My only suggestion is that for ER processing Step 3. 30 min incubation, have you thought about using a thermomixer (to mix and/or heat?)

#### Response

following added to seciton 5.2: "Note: SUPNAT and MISC samples to be stored frozen for 1

#### fixed

Yes I thought about it, but was thinking would start with the equipment we currently have and see if it works. If we need to tweak the method to get it to work better, then this is definitely an option

Manager	Feedback	Response
AKL	Minor text change: Section 5.1.2: "The number of samples required is dependent"	Amended as per suggestion
	Page 7 Second paragraph 'Note' refers to further testing with the new batch of AP and	Amended as per suggestion
AKL	frozen samples – can we add some detail covering the outcome of this further testing	
PMB	Title: 'Pt 4 additional testing' instead of 'Pt 4 amended'	Amended as per suggestion
	'Introduction' expanded: refer to "additional testing" and add "a modification to the	Amended as per suggestion
	previous experiments is proposed in this document. Note: "Testing completed" and	
PMB	"Additional testing" is defined within the body of this document."	
	3.3 'Reporting': Updated to include reference to meetings with Senior Scientist Quality and	Amended as per suggestion
	Projects team and also that Draft and Final Project reports are to be provided to the Decision	
PMB	Making Group for Review	
DMAD	6.2i 'Intent': identify 'experiment 4, part 1' when talking about the 'poor presumptive	Amended as per suggestion
PMB	results'	ADM: Mith respect to the lest point "if all good then us'll validate" It may well be that we
	<ol><li>Results and Data Compilation: Suggested add: 'If results are acceptable, full validation of the new process will ensue.'</li></ol>	ARM: With respect to the last point "if all good, then we'll validate" It may well be that we have performed sufficient work to call it validated from what we have done in the project?
	the new process will ensue.	Can we word it something like "if all good, then consideration of whether additional testing
		is required for validation will occur, and if required supplementary testing will be
		performed in order to meet validation requirements" ? PMB "Happy with that wording."
PMB		performed in order to indeer validation requirements.
KDR	Looks good to me I have happy to sign off on this document as written	NA
JAH	I have no feedback.	NA
LBR	All good from me.	NA
	I'm having trouble assessing this proposal as I have no recollection of what has been tested,	
	what the results were and why any further testing is required. I know that there are some	
	results that haven't turned out the way they were expected and that this further testing was	
	to try to fill the gap for a process to be used all the time. However without having a good	
	understanding of the problem encountered, I don't know how to assess if these further	
	proposed tests are going to help. I would have liked some sort of short summary document.	
	Perhaps this has been written already. If so Please direct me to where. I haven't looked due	
	to the time pressures to give feedback. I'd love to better understand what the big picture	Man II
CNAL	workflow is. What are we trying to achieve by changing the workflow and what is expected	
SMJ	from the changes.  4b iii - AP positive / p30 positive submit for quantification and amplification, if Epithelial	Reports to SMJ, and discussed latest results.
	fraction was originally marked as "Extract and hold", then submit Epithelial fraction for	
	quantification and amplification as well. If the accompanying sp fraction is Ap pos & p30	Given pos for poss seminal fluid, we have some evidence that there may be male cells. It is
	pos, is there a need to remove these from Extract and hold? This need may be different in	possible that there are only epis from the suspect and therefore the epi fraction will be
	this test environment to the need in practise.	profiled.
	5.1.1 – Does the standard procedure use neat semen for pos controls? Is this bit just trying	
	to say that the dilutions differ from the controls or is it the difference between using neat	standard procedure uses a 1/10 dilution semen for diff controls. No-one in evidence
	semen vs something else. When using neat semen, wasn't there a worry about the hook	recovery has ever recalled seeing the high dose hook effect in the lab we have never
	effect for p30?	even seen it with neat semen when testing p30 kits
	5.1.1 – approximately 3 times the amount of epi cells will be added 3 times what? The	3 x times the epi cells compared to a standard diff control – this was an attempt to
	number of sperm?	replicate the casework samples with lots of epis
KDR	Awaiting feedback	

Date Person 2/07/2020 KDR	Feedback	response	date person 2/07/2020 ARM	method e-mail
Z/U//ZUZU KUK	Could you please include Emma in this email given:	We discussed as a team who should be listed as authors when we were drafting the final report, and we considered whether to include Emma, but had decided not to at that time because she had not had any input into the conclusions and recommendations and felt that it was unfair that we included someone as an author to	2/01/2020 AKM	C-111011
	<ul> <li>that she is listed under project personnel</li> <li>she was working with you on the project from August 2016 until she went off sick last year – almost 3 years</li> </ul>	a document that they did not write and may not necessarily agree with the conclusions or recommendations		
	- her name is on all of the project plans	reached. However we also see that it is unfair to not include someone who had a significant input to the project as well. We had attempted to address this elsewhere in the document, but we accept that it is better to list		
	I would also propose that her name is included on the cover page of the final report.	Emma amongst the authors, and as such we will make this change along with any other changes that may be required prior to finalisation.		
		We will be changing the authorship from:		
		Allan McNevin, Matthew Hunt, Chelsea Savage, Kirsten Scott, Paula Brisotto, Cathie Allen to:		
	Given this size and length of this project, it has been presented with spectacular clarity.	Matthew Hunt, Allan McNevin, Chelsea Savage, Emma Caunt, Paula Brisotto, Cathie Allen		
	Concepts have been explained concisely - with supporting data provided in a digestible format. Thankyou it makes it a pleasure to read			
	Minor feedback:			
	■Typo page 57: Microscopy, dot point 1 "seeTable" — add a space  ■Typos page 57: AP, dot point 1 "seeTable" — add a space	Typos fixed		
3/07/2020 KDS	<ul> <li>Did you want to recommend that a higher volume of supernatant is added to p30 tests routinely?</li> <li>Firstly – congratulations to all of you for getting this project finalised.</li> </ul>	Volume of supernatant added to p30 will be assessed as a subsequent project / process to this	22/07/2020 ARM	e-mail
	The report is excellent, reads really well and is very easy to follow and understand. This is a considerable achievement given the length			
	of the project and the number of experiments conducted. Really well done.			
7/07/2020 LBR	I don't have any changes or suggestions other than the TOC needs to be fixed.  I dreaded the thought of having to read this report considering the amount of work and length of time this project has endured,	TOC fixed	N/A N/A	N/A
8/07/2020 ALL	however I was pleasantly surprised at how easy it was to read, the results, discussions and conclusions all made sense. And that is my feedback	N/A	N/A N/A	N/A
	I have very little feedback to give on this report and given the size of it you all should be congratulated for your efforts! As a whole the report is written extremely well Matt. Excellent work! I found it really easy to read and I really liked the way the whole project was			
16/07/2020 SMJ	stitched together by looking at the results at each step and then explaining the reasoning behind the direction taken for continued testing. I have a few minor edits to suggest but I'm happy with the overall document	N/A	N/A N/A	N/A
16/07/2020 SMJ	Part 15.1 last paragraph: I found the repeated use of ~ for approximately a bit distracting. Perhaps some are not needed or the word could be used occasionally	replaced some ~ with "approximately"	22/07/2020 ARM	N/A
	For tables 1 2 & 3 in particular I struggled to make comparisons between the dilutions easily and I think that these tables could benefit from a solid line divide between each dilution horizontally. The other smaller tables are fine but I know having the setup differ for just			
16/07/2020 SMJ	a few is likely to do a certain someone's head in, so it could be considered for all.	lines added for tables 1 & 2	22/07/2020 ARM	N/A
16/07/2020 SMJ	5.6 Microscopy 5 <sup>th</sup> bullet point: "Even for the least dilute replicates ('1/50')" Should this not be 1/5? As i/50 is not the least dilute 5.6 Microscopy 6 <sup>th</sup> bullet point: "These occurred at lower concentrations of semen dilution". I found this combo of words confusing.	point removed, unclear what was intended by this discussion point	22/07/2020 ARM	N/A
16/07/2020 SMJ	Are you trying to say lower concertation of semen Or less diluted semen? I suggest perhaps removing the word "dilution" and adjust the term to suit with the concentration of semen	reworded to " occurred at lower concentrations of semen (higher dilutions) and"	22/07/2020 ARM	N/A
16/07/2020 SMJ	adjust the term to suit with the concentration to senier  6. Last sentence: add "be" those heads may be expected to"  Tables 31-44 Again I think they would be slightly easier to read if there was a solid divide vertically between the current method and	suggestion incorporated	22/07/2020 ARM	N/A
16/07/2020 SMJ	the proposed method. I think it would help to train the eye to what it is you are comparing.	suggestion incorporated  We were adding 150uL to the kits, as per the SOP. The proposed method retains the p30 prior to Analytical	22/07/2020 ARM	N/A
	11.3 p30 results 1 <sup>st</sup> para page 54 – you talk about an issue with the p30 kits and that it should be noted the manufacturer recommends 200uL be added. In the method tested, how much fluid was added? I know we were trying to balance between too dilute and having	we were adoing 150ut to the kits, as per the SUP. The proposed method retains the p30 prior to Analytical processing, at which point the Phadebas portion will be retained (or not) depending on the extraction method requested. As discussed over the phone, at times 150ut isn't enough to ensure the sample migrates sufficiently		
16/07/2020 5841	enough for all presump testing. How much short of 200uL is it if you need to presump test both p30 & Phadebas?	along the test strip and a further 50 uL is required. I would like to investigate this further, but don't want to have	22/07/2020 ADM	e-mail /
16/07/2020 SMJ	11.4 last para (just above conclusions): Please remove "Eventually" from the beginning of the last sentence and change to just "It was	the project go off on a tangent, so will do so separately	22/07/2020 ARM	verbally
16/07/2020 SMJ	decided" no matter how long it took to make the decision (a)  2nd Recommendation: Suggest adding to the end of the sentence "in the absence of spermatozoa" as I see no need to presump test	word "eventually" replaced with "ultimately"	22/07/2020 ARM	N/A
16/07/2020 SMJ	anything where sperm has been confirmed via micro  Lastly, I thought that there should be some sort of direction mentioned to indicate workflow or just summarise workflow. So what I	suggestion incorporated	22/07/2020 ARM	N/A
	take from this is that all samples expected that could have seminal fluid on them are submitted for diff and that the slides from the diff are checked for sperm. No ER slides are to be prepared. Is that correct? If so, are the results of the micro going to guide us any further			
	with the testing conducted other than potentially testing the S/N for p30? Are we to continue to keep the E fracs on hold? Do we need to add something into our statements to address problems we've had before to explain why a diff was done when there were no sperm			
16/07/2020 SMJ	found? Abstract comment:	Recommendations given numbers, Appendix 2 created which summarises workflow for recommendation 1.	22/07/2020 ARM	e-mail
	"Perhaps better wording here to the effect of: 'undertaken in an attempt to investigate if the sensitivity of spermatozoa microscopy could be improved."			
15/07/2020 JAH	"semen microscopy" change to "spermatozoa microscopy" and "Diff Lysis" updated to "Differential Lysis (Diff Lysis)"	suggestions incorporated	22/07/2020 ARM	N/A
	Introduction comments: "staff from the Forensic Reporting and Intelligence Team (FRIT) raised concerns regarding" to be deleted			
	"which showed" reword to "were observed to have" "following" reword to "during"			
15/07/2020 JAH	"even to the extent of observing 3+ or 4+ sperm heads" reword to "some with observations of 3+ or 4+ sperm heads" "our clients" reword to "the client"	suggestions incorporated	22/07/2020 ARM	N/A
15/07/2020 JAH	Introduction question: "This is sometimes referred to as stochastic effects." - is there a reference for this	No, comon usage	N/A N/A	N/A
	Section 5.1 comment: "Primary concerns relating to the sensitivity of ER slide microscopy led to two initial questions being raised:" reword to "To investigate		,	
15/07/2020 JAH	the sensitivity of the ER slide microscopy process, two questions are posed:" section 5.6 comments:	suggestion incorporated	22/07/2020 ARM	N/A
	Microscopy:  comment on second last dot point "Even for the least dilute replicates ("1/50"), the highest observed sperm head count was only 1+." -			
	Is there a need for this point? If so, it would need to be indented as it follows the point above. It would need rewording for clarity:  'least dilute replicates where sperm was detected (in each replicate)'			
	reword last point to "spermatozoa were observed on the Diff Lysis slides ('very hard' or 'hard' to find)."	Microscopy:		
	AP and p30	second last dot point removed as per feedback to SMJ; wording suggestion for last dot point incorporated		
15/07/2020 JAH	Ar airu usu "330 Sensitivity limit could not be established (two of the four replicates gave a negative result at '1/50' dilution)" - Wouldn't the limit be 1/10 based on definition of sens limit in 5.3?	AP & p30: to 1/10 sensitivity limit corrected	22/07/2020 ARM	N/A
15/07/2020 JAII	Section 5.7 suggestions: "infrequently" reword to "in low numbers"	Sensitivity mint confected	22/07/2020 AKW	N/A
	"The conditions under which the issue was observed in casework not being replicated in this experiment" reword to "The conditions			
	under which the differences were observed in casework not being replicated in this experiment"			
	Question: "Experimental mock samples not being truly representative of casework samples" - Does this point relate to the dot point 2 here? I			
	guess the 'conditions' in that point relate to the mock samples not being truly representative eg. using buccal cells rather than vaginal cell suspension?			
	suggestion:	suggestion incorporated		
	"Design further experiments to investigate whether there are possible issues with the current ER method" reword to "Design further experiments to investigate whether there are ares to improve with the current ER method"	regards to question: yes the point relates to the fact the mock samples are not exactly the same as casework, and		
15/07/2020 JAH	insert the word "some" to "If no significant issues with the current method can be identified, then the observations in some casework regarding differences in microscopy sensitivity are not easily explainable"	there may be factors in the sample type that effect the processing of casework samples that we are not able to replicate in testing with mock samples	22/07/2020 ARM	e-mail
15/07/2020 JAH	Section 6.1 - wording suggestion as per SMJ Section 6.2 wording suggestion addition of work "separate" "2.Supernatant and spin basket swab were transferred to new (separate)	suggestion incorporated	22/07/2020 ARM	N/A .
15/07/2020 JAH 15/07/2020 JAH	tubes, leaving the cell pellet and a small amount of supernatant to be resuspended. " Section 7.4, second paragraph P30 corrected to p30	suggestion incorporated correction made	22/07/2020 ARM 22/07/2020 ARM	N/A N/A
	Section 9.1 Suggest reword "As no positive AP results had been obtained for the proposed method in the previous experiment, the sensitivity of			
15/07/2020 JAH	the method was of concern." to "As no positive AP results had been obtained for the proposed method in the previous experiment, the sensitivity of the method required further investigation."	suggestion incorporated	22/07/2020 ARM	N/A
	Section 10.1 suggestion " the poor performance of the AP test was still concerning, and this prompted a question to be raised as to whether excess dilution			
	may be affecting AP detection sensitivity. " reword to " the poor performance of the AP test still required further investigation, specifically on whether excess dilution may be affecting AP			
15/07/2020 JAH	detection sensitivity. "	suggestions incorporated	22/07/2020 ARM	N/A
-, - ,				

	11.4 Part 7 discussion comment: "Although the majority of AP tests showed equivalent detection sensitivity between the proposed and current methods, there were still more samples for which the current method gave superior results than samples for which the proposed method was better. Overall AP			
15/07/2020 JAH	testing produced quite poor results using either method. This was one area where the proposed method did not perform satisfactorily, an issue which has been highlighted consistently throughout this project." an issue which has been highlighted consistently throughout this project - I don't think this part is necessary for the point	I think the point is trying to be made that this was consistent finding, reworded to "This was one area where the proposed method did not perform satisfactorily, and this was consistent throughout this project."	22/07/2020 ARM	e-mail
	12. Conclusions - suggestions: "This project was initiated in response to concerns regarding a small number of casework samples" reword to "This project was initiated to investigate the observations in a small number of casework samples" "Although this issue did not affect all samples equally" reword to "Although these observations did not affect all samples equally"			
	" semen microscopy at ER nay be inadequate for the consistent detection of low numbers of spermatozoa." reword to " semen microscopy at ER could potentially be improved."  " it is desirable for microscopy to be optimised for maximum possible sensitivity in order to be able provide the most informative results." reword to " it is desirable for microscopy to be optimised for maximum possible sensitivity in order to be able to provide the			
	most informative results." "When consulted, FRIT staff members were generally of the opinion" reword to "When consulted, court reporting scientists were generally of the opinion"			
15/07/2020 JAH	FRIT to be removed from Abbreviations as not used in the document  Abstract:	suggestions incorporated (FRIT left into abbreviations as it is found in sign-off part of document) Wording following previous feedback was changed to: "This project was undertaken in an attempt to investigate if the sensitivity of spermatozoa microscopy could be improved." now updated to "This project was undertaken following observations from staff where on occasions differences	23/07/2020 ARM	N/A
	Reword the first part to "This project was undertaken in response to staff queries regarding the sensitivity of semen microscopy of items from sexual assault cases due to differences occasionally observed between microscopy slides prepared at the examination	were seen between microscopy slides prepared at the examination compared to those prepared during DNA extractions process. This project then investigated if the sensitivity of spermatozoa microscopy could be		
17/07/2020 PMB 17/07/2020 PMB	compared to the DNA extractions process." Abstract: "Diff Lysis" updated to "Differential Lysis (Diff Lysis)"	improved.**  change already incorporated	23/07/2020 ARM N/A N/A	N/A
	Introduction 2.1 Background information Suggestion to reword first paragraph to: "In 2015, staff from within Forensic DNA Analysis raised the notion that initial slide microscopy	Wording previously updated to "In 2015, a small number of sexual assault casework samples were observed to		
17/07/2020 PMB	conducted during the Evidence Recovery examination process may have a lower sensitivity than the slides produced during the differential lysis extraction process, presenting as a marked difference in the spermatozoa microscopy counts for a small number of sexual assault cases."	have marked differences between the original spermatozoa microscopy count obtained during Examination by the ER Team and a subsequent count from microscopy slides prepared during the Diff Lysis Extraction procedure." -	23/07/2020 ARM	N/A
	Introduction 2.1 Background information, suggest addition to the final paragraph  "should this be the only sample submitted. Generally, multiple subsamples are submitted for this reason, and crime scene samples contain unknown amounts for DNASome more wording in here about – generally multiple sub-samples submitted from a larger	wording adjusted to: "Failure to detect spermatozoa due to limitations in microscopy technique or sensitivity, may carry serious negative implications for the effective investigation of sexual assault casework, should the affected sample be the only sample submitted. Generally multiple samples are submitted for SAIKs, and often		
17/07/2020 PMB	item, or multiple swabs from a SAIK. Samples still go through cell extraction, case assessment . Etc etc." 5.1 suggest rewording "Primary concerns relating to the sensitivity of ER slide microscopy led to two initial questions being raised." to	more than one sub-sample is collected from a larger item, thus somewhat reducing the overall risk to case."	23/07/2020 ARM	N/A
17/07/2020 PMB	"Primary queries relating to the sensitivity of ER slide microscopy led to two initial questions being raised:" 5.1 point I. suggest reword from "I.Is there an issue associated with the ER slide staining procedure, such that spermatozoa" to	wording from JAH incorporated	N/A N/A	N/A
17/07/2020 PMB	"I.Was the ER slide staining procedure performing sub-optimally, such that spermatozoa"  5.7 suggest reowdring "Following the review of the experimental data, the following two options were considered:  1. Design further experiments to investigate whether there are possible issues with the current ER."  to "Following the review of the experimental data, the following two options were considered:	Suggestion incorporated	23/07/2020 ARM	N/A
17/07/2020 PMB	1.Design further experiments to investigate whether the current ER method was performing sub-optimally." 5.7 Question: "If no significant issues with the current method can be identified, then the observations in casework regarding differences in	rewording from JAH already incorporated  The background part of the document does provide some context to this statement regarding the expected differences, whereby it is acknowledged that a "A moderate increase in the concentration of spermatozoa from	N/A N/A	N/A
	microscopy sensitivity are not easily explainable. Irrespective of the cause of this issue, these observations are significant enough that a change to current ER processing is still recommended;" Question: Rewording? While there will always be outliers in any process (due	ER microscopy to Diff Lysis microscopy is not unexpected"		
	to the substrate, the conc factor, stochastic effects etc etc) there will always be an expected difference in the concentration between ER and D/L So, the question was really – is the expected difference between ER and D/L acceptable? (could be up to 10 fold if 300uL in ER	samples with marked differences in microscopy sensitivity are not easily explainable. Irrespective of the cause of		
17/07/2020 PMB	went to 30uL at D/L).  Or, should we investigate a more sensitive method using the D/L step as continuous improvement?	this issue, these observations are significant enough that a change to current ER processing is still worthy of investigation;"  This information was taken from Project #181 Interim report #1 v1.1 (last dot point on page 3). It wasn't	23/07/2020 ARM	e-mail
	5.7 Question: "As the problematic samples represent less than 10% of samples where no spermatozoa are observed from an ER slide" - Where is	recorded where this data was from at that time, although the feeling is that it was a from a review of data from the samples which had undergone the interim process of being extracted regardless of initial sperm microscopy		
17/07/2020 PMB	this info from?  Section 7.2, suggestion to reword from "Based on the results of previous testing, semen dilutions approaching the Limit of Detection	and had the diff slide read as a matter of course	23/07/2020 ARM	e-mail
17/07/2020 PMB 17/07/2020 PMB	(LOD) were selected, to provide the most. "" to "Based on the results of previous testing, semen dilutions approaching the Limit of Detection (LOD) for their respective screening tests were selected, to provide the most" Section 7.4, second paragraph P30 corrected to p30	suggestion incorporated correction made from JAH feedback	23/07/2020 ARM N/A N/A	N/A N/A
17/07/2020 PWIB	Section 7.4, second paragraph P30 Corrected to p30 Section 9.1 Suggest reword "As no positive AP results had been obtained for the proposed method in the previous experiment, the sensitivity of	conection made iron pan recorder.	N/A N/A	NA
17/07/2020 PMB	the method was of concern." to "As no positive AP results had been obtained for the proposed method in the previous experiment, the sensitivity of the method required further investigation."	suggested rewording identical to JAH feedback already incorporated	N/A N/A	N/A
17/07/2020 PMB	9.4 Part 5 discussion, grammer correction "Sensitivity" to "sensitivity"  10.1 Part 6 - Purpose and Scope, suggest rewording: "After reviewing the results obtained in the previous experiment, the poor	suggestion incorporated	23/07/2020 ARM	N/A
17/07/2020 PMB	performance of the AP test was still concerning, and this prompted a question to be raised as to whether excess dilution may be affecting AP detection sensitivity." to "After reviewing the results obtained in the previous experiment, the poor performance of the AP test prompted a question to be raised as to whether excess dilution may be affecting AP detection sensitivity."	Wording from JAH previously incorporated, updated to "After reviewing the results obtained in the previous experiment, the poor performance of the AP test required further investigation, specifically on whether excess dilution may be affecting AP detection sensitivity."	23/07/2020 ARM	N/A
,,	11.4 Part 7 discussion, suggestion: reword from "The finding that Diff Lysis microscopy gave slightly superior results when compared to the proposed method microscopy		,,	
	(4 times out of 26) was not considered to be overly significant, given these methods" to "The finding that Diff Lysis microscopy gave slightly superior results when compared to the proposed method microscopy (4 times out of 26) was not considered to be overly		23/07/2020 ARM	
17/07/2020 PMB	substantial, given these methods"  11.4 Part 7 discussion, suggestion: reword last sentence: to "This was one area where the proposed method did not perform satisfactorily, a trend which has been	suggestion incorporated  feedback from JAH resulted in some rewording, further modified to "This was one area where the proposed	23/07/2020 ARM	N/A
17/07/2020 PMB	highlighted consistently throughout this project."  Reword "There did not appear to be significant differences between the results obtained" to "There did not appear to be substantial	method did not perform satisfactorily, and this was a consistent trend throughout this project."	23/07/2020 ARM	N/A
17/07/2020 PMB	differences between the results obtained" as use of 'significant' differences often requires stats  12. Conclusions: first paragraph	suggestion incorporated  rewording from IAH already incorporated to "This project was initiated to investigate the observations in a small	23/07/2020 ARM	N/A
	12. Concusions, ints paragraph  Suggestion to reword first sentence to "This project was initiated in response to noted differences in sensitivity between ER and Diff  Lysis microscopy for a small number of casework samples for which zero sperm had been observed at ER microscopy, despite  subsequent Diff Lysis microscopy showing sperm heads easily observable at that stage."	rewording from JAH aiready incorporated to 1 inis project was initiated to investigate the doservations in a small number of casework samples for which zero sperm had been observed at ER microscopy, despite subsequent Diff Lysis microscopy showing sperm heads easily observable." also already reworded to "Although these observations did not affect all samples equally, it drew attention to		
17/07/2020 PMB	of conducting semen microscopy at ER may be an area for improvement to allow a more consistent detection of low numbers of spermatozoa."	the fact that the current practice of conducting sensors increases in the fact that the current practice of conducting sensors in increases at an angle sequelly, it were waterfall for the adjusted to "active the fact that the current practice" conducting sensors in increases an angle sequelly in the improved." further adjusted to "active the fact that the current practice of the fact that the current practice	N/A N/A	N/A
17/07/2020 PMB	12. Conclusions, second paragraph, reword "mitigation measure" to "modified process" 12. Conclusions, second paragraph,	suggestion incorporated	23/07/2020 ARM	N/A
	Comment on sentence "Often microscopy results obtained after Diff Lysis were incongruent with the sperm count on initial ER slides." - 'Often' is not correct. The majority were congruent, zero detected at ER and Zero detected at D/L. The second most common tended to			
17/07/2020 PMB	be 0 ER to <+1 (expected). There were some differences noted, however I wouldn't go into this here, as that data assessment is not part of this project.	"often" removed from wording "Microscopy results obtained after Diff Lysis were, at times, incongruent"	23/07/2020 ARM	N/A
	12. Conclusions, third paragraph, reword from "Initial investigations into the possible cause of reduced sensitivity of ER semen microscopy were inconclusive and exaggerated differences between ER and Diff Lysis microscopy were not able to be replicated." to "Initial investigations into the			
17/07/2020 PMB	possible cause of the notable difference in sensitivity of ER semen microscopy compared to Diff Lysis microscopy were inconclusive and exaggerated differences between ER and Diff Lysis microscopy were not able to be replicated."	suggestion incorporated	23/07/2020 ARM	N/A
17/07/2020 PMB	5th paragrpah suggest replacing "concerning" with "problematic"  7th paragraph, Question relating to: "The inability to differentiate spermatozoa types if the proposed method is adopted is therefore	suggestion incorporated  Question has been noted, no change to the report but it may form part of implementation plan – i.e. if DPP say no, oroiect cannot be implemented and alternatives would need to be proposed and tested – but under a new	23/07/2020 ARM	N/A
17/07/2020 PMB	In paragraph, Question relating to: I ne inability to differentiate spermatozoa types it the proposed method is adopted is therefore not expected to meaningfully impact upon the service provided to our clients." - Does this need to be proposed to DPP?	no, project cannot be implemented and alternatives would need to be proposed and tested – but under a new project number	23/07/2020 ARM	e-mail
	Comment on 12. Conclusions, 7th paragraph "The presence of whole sperm as distinguished from sperm heads is not currently reported within the standard Statement of Witness, and this topic is only rarely discussed as part of expert testimony. The inability to			
	differentiate spermatozoa types if the proposed method is adopted is therefore not expected to meaningfully impact upon the service provided to our clients." Comment: I think this is a question for ALL reporters. I know time since deposition can be wishy washy evidence but, in some cases, it could help. I was originally trained to consider heads and tails and time since deposition and whilst I			
	evidence but, in some cases, it could nep, i was originally dailled to closure friends and cans and time since deposition and winner in haven't reported such opinion in ages, I am worried about removing the ability to do so. I am reluctant to implement a change like this without all of our reporters approval. The following paper by Dziak et. Al. suggest some value:			
	Providing Evidence Based Opinions On Time Since Intercourse (TSI) Based On Body Fluid Testing Results Of Internal Samples			
	Renata Driak, Linda Parker, Vanesas Collins & Sarah Johnston Pages 59-69   Published on line: 22 Nov 2013 A critical evaluation of the current available literature on Time Since Intercourse (TSI) was performed at the Centre of Forensic Sciences			
	(CFS) to determine whether there is scientific support for reliable, evidence based opinions on TSI. The assessment included a review of published scientific literature and internal studies focusing on the persistence of spermatozoa, prostatic acid phosphatase and prostate			
	specific antigen (PSA/p30) from internal samples of living individuals. From this review, it was concluded that, despite variation in sampling methodologies, there is scientific support for the development of TSI estimate guidelines based on the serological testing	With respect to the potential loss of the ability to comment on Time Since Intercourse (TSI) based on the presence or absence of intact spermatozoa, this was identified earlier in the project's lifecycle. The proposed		
	results from internal swabs and smears.  A more reliable way to consider time since deposition is through RNA degradation – and so if we were able to get this implemented in	process was approved for further experimentation based on that understanding. Whilst it is an important consideration, agreement has been reached that sufficient experimentation has been carried out and this project can be finalised. We propose that this point be raised with respect to implementation subsequent to finalisation		
23/07/2020 KDR	A more reliable way to consider time since deposition is through RNA degradation – and so if we were able to get this implemented in the lab, that may allay possible concerns about removing this service.	can be finalised. We propose that this point be raised with respect to implementation subsequent to finalisation of the project report.  End of the paragraph reworded to: "Anecdotally, ER scientists have stated that in their experience, this		
		phenomenon has not been observed at Forensic DNA Analysis. In order to produce a high dose hook, samples need to replicate the levels seen when neat semen is applied to the test device. Exhibits with visible stains		
		similar in appearance to semen, with subsamples where no spermatozoa are observed on microscopy (i.e. an aspermic semen stain is suspected), can be re-tested with a dilution made from the retained suspension.  Therefore, the high dose hook effect is therefore not expected to preclude the sole use of p30 as a screening		
23/07/2020 KDR	would be far in excess of the amount typically present in human semen. *Comment: is there some evidence to support this claim (other than anecdotal)?	Interence, the night dose nook effect is therefore not expected to preclude the sole use of p.su as a screening tool."		

# Cass Forensic and Scientific Services

# **Investigating Adverse Events in DNA Analysis**

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#### 1. PURPOSE

The purpose of this document is to provide guidelines around standard investigation protocols and result acceptance criteria for adverse events in the DNA Analysis Unit. The procedure outlines the key considerations in an investigation, the required actions and the necessary documentation for issues that may interfere with the quality of results within the DNA Analysis Unit.

These guidelines have been developed in complement to the OQI process (QIS <u>13965</u>), and the Procedure for Quality Practice (QIS 17154).

#### 2. SCOPE

This procedure applies to all staff within the DNA Analysis Unit. This document has attempted to cover key quality issues that may arise from adverse events in sample preparation, in screening of exhibits for biological fluids and in DNA profiling; however it can not cover all possible adverse events. Where an event occurs which is outside the scope of this document, consult Senior Scientists and Team Leaders for guidance. This document does not cover adverse events that relate to workplace health and safety.

### 3. **DEFINITIONS**

For a comprehensive list of abbreviations refer to QIS <u>23849</u> Common DNA Analysis Terms and Acronyms.

Adverse Event: Any event or occurrence which brings into question a procedure or result

AI: Allelic imbalance

AP: Acid phosphatase

**CE**: Capillary electrophoresis

**DNA Profiling techniques:** All procedures, analytical instruments and consumables used in the process of obtaining a DNA profile (including extraction, quantification, amplification, capillary electrophoresis and profile interpretation).

**EB Check**: Extraction batch check completed by reporting staff as a quality check for adverse events occurring during the automated DNA extraction process.

**EFTA:** Extraction FTA sample

**OQI:** Opportunity for quality improvement

ReGs (Re-CE): Sample or batch is re-prepared and analysed again on the 3130x/

SD: Standard deviation

TMB: Tetramethylbenzidine

**QEXH:** Case management list to hold quarantined samples

**QPS:** Queensland Police Service

# 4. EVIDENCE RECOVERY - PRESUMPTIVE TESTING QUALITY CONTROL

#### 4.1. Tetramethylbenzidine (TMB) presumptive screening

Tetramethylbenzidine (TMB) is a presumptive test for blood used within the DNA Analysis Unit. Before the reagent can be used for casework, both positive and negative controls must pass quality control criteria.



The positive TMB control is a known blood sample (Refer to QIS 17190 for testing methodology). A positive control pass is the appearance of the blue-green colour developing in <5 seconds. A colour change in 5-20 seconds should be considered inconclusive and the test repeated, if after repetition it is still inconclusive this should be considered a fail (refer below). A positive control fail is the absence of the blue-green colour change, or the appearance of the blue-green colour developing >20 seconds. If a colour change occurs after the addition of TMB only (without hydrogen peroxide) it is also a failed test.

The negative control for TMB (Refer to QIS <u>17190</u> for testing methodology) is performed on a substrate that does not react to TMB (e.g. clean filter paper). A negative control pass is the absence of a blue-green colour developing in 10 seconds, with a negative control fail being the development of a blue-green colour within 10 seconds.

If the positive or negative controls fail, the TMB and hydrogen peroxide reagents should be re-prepared and the controls re-tested (Refer to QIS 17190 for methodology). If the new reagent preparation has passing TMB controls, the chemical test is acceptable for use. If the newly prepared reagents fail the quality criteria for TMB positive and negative controls, notify the Senior Scientist Evidence Recovery. New reagents may need to be purchased and new positive controls prepared for testing.

# 4.2. Acid Phosphatase (AP) presumptive screening

Acid phosphatase (AP) is a presumptive test for seminal fluid used within the DNA Analysis Unit Laboratory. Before the reagent can be used for casework, both positive and negative controls must pass.

The positive AP control is a known seminal fluid sample (Refer to QIS <u>17186</u> for testing methodology). A positive control pass is the appearance of a purple colouration within 5 seconds. A positive control fail is purple colouration developing >5 seconds or the absence of the purple colour change after 5 seconds.

The negative control for AP (Refer to QIS 17186 for testing methodology) is performed on a substrate that does not react to AP (e.g. clean filter paper). A negative control pass is the absence of a purple colouration within 2 minutes. A negative control fail is the development of a purple colouration within 2 minutes.

In cases where the positive or negative controls fail, the AP reagent should be re-prepared and the controls retested (Refer to QIS <u>17186</u> for methodology). If the new reagent preparation has passed AP controls, the reagent is acceptable for use. If the newly prepared reagent fails the quality criteria for AP positive and negative controls, notify the Senior Scientist Evidence Recovery. New reagents may need to be purchased, and new positive controls prepared for testing.

#### 4.3. Phadebas presumptive screening

Phadebas is a presumptive test for saliva used within the DNA Analysis Unit. The laboratory utilises both a supernatant (liquid), and a paper based testing procedure (Refer to QIS 17193 for methodologies). Positive and negative controls must both pass for the test results to be accepted and reported.

The phadebas positive control is a known saliva sample (obtained from staff), and the negative control is a Nanopure water only sample. The positive and negative controls for the supernatant test are different from those used in the paper based test, refer below for details.



### Phadebas paper test:

A positive control pass is the development of pale blue zones on the blank side of the phadebas paper at 40 minutes. On the spotted/treated side of the paper the blue spots appear dissolved or smudged (Refer to QIS 17193 for methodology). A positive control fail would be the absence of the pale blue zones on the blank side of the paper, and/or the absence of the dissolved/smudged blue spots on the treated side at 40 minutes.

A negative control pass is indicated by no colour change on either side of the paper (at 40 min.), with a negative control fail occurring if the phadebas paper develops pale blue zones or dissolved/smudged areas on the treated side of the paper at 40min. (Refer to QIS 17193 for methodology).

For phadebas paper based testing, the positive and negative controls should be processed prior to use on casework samples, as a control failure would constitute an unacceptable risk to the exhibit.

If a positive control fails for the phadebas paper, the paper should be retested with the saliva of a different staff member used as the positive control (Refer to QIS 17193 for methodology). If the second control passes, the results can be accepted (as individual staff may have differing levels of amylase). If the second positive control does not pass, notify the Senior Scientist Evidence Recovery, as new phadebas paper may need to be purchased.

If the negative control for the phadebas paper test fails (Refer to QIS 17193 for methodology), fresh nanopure water should be obtained and the negative control retested. If the retested negative control passes, the phadebas test can be performed on casework samples. If the negative control still fails an investigation will be required. The investigation should consider the area in which the test was performed (e.g. laboratory bench), the equipment (spray bottles) and the water used for possible contribution of amylase and/or the function of the phadebas paper. Casework samples are not able to be processed until both the positive and negative controls pass.

## Phadebas supernatant test:

A positive control pass is indicated by a blue coloured supernatant in the positive control sample after processing (Refer to QIS <u>17193</u> for methodology), with a positive control fail indicated by the absence of a blue colouration in the supernatant.

A negative control pass is indicated by a clear and colourless supernatant in the negative control sample after processing, and a negative control fail occurring if the supernatant is blue in colour (Refer to QIS 17193 for methodology).

In the phadebas supernatant testing procedure a positive and negative control are processed prior to casework samples being tested, this ensures that the reagents are suitable for use (i.e. reagent controls).

If the positive control fails for the phadebas supernatant test, it should be retested with the saliva of a different staff member (Refer to QIS 17193 for methodology). If the second control passes, the results can be accepted (as individual staff may have differing levels of amylase). If the second positive control does not pass, in consultation with the Senior Scientist Evidence Recovery an investigation may be required and/or new phadebas tablets may need to be purchased.



If the negative control for the phadebas supernatant test fails (Refer to QIS 17193 for methodology), in consultation with the Senior Scientist Evidence Recovery and Senior Scientist Quality and Projects an investigation should be initiated. The investigation should examine the environment, processing procedure, labware and reagents used in testing for possible sources of amylase. Until the positive and negative reagent controls pass, no casework samples can be processed.

If the first set of controls pass (reagent controls), the positive and negative controls are rerun with the casework samples (as methodology controls). On completion of the batch the pass/fail status of the controls determines if the casework phadebas results can be accepted and reported.

## 4.4. ABAcard p30 - seminal fluid presumptive screening

ABAcard® p30 test (Abacus Diagnostics Inc.) detects p30 and is a presumptive test for seminal fluid used within the DNA Analysis Unit. The ABAcard® device has two result areas within the device window; the control "C" area and the test "T" area.

On completion of the test, a pink line in the "C" area is a positive control pass, and indicates that the test is functional. On test completion a pink line in the "T" test result area is a positive test result i.e. presumptive positive for seminal fluid. The absence of a pink line in the "T" test area is a negative test result i.e. presumptive negative for seminal fluid.

For valid use of the ABAcard® test, the positive control line must be apparent on completion of the test, and the test must not be used after the expiration date. If there is no pink line visible in the "C" control area of the test, it is inconclusive and the test should be repeated. If the second ABAcard® test fails (i.e. no pink line visible in the control area) notify the Senior Scientist Evidence Recovery. New test kits may need to be purchased.

# 5. SAMPLE PREPARATION/PROCESSING - ADVERSE EVENT INVESTIGATIONS

Adverse events can occur during sample preparation and/or processing. This procedure is not able to provide a comprehensive coverage of all possible adverse occurrences, but will outline the three most critical types of events which may occur and would require investigation. These include:

Incorrect labelling (Refer <u>section 5.1</u>)
Sample cross contamination (Refer section 5.2)
Incorrect use of reagents (Refer <u>section 5.3</u>)

Minor adverse events, adverse events which do not require corrective actions and/or adverse events which do not require investigation must be detailed in specimen notes (e.g. mis-storage of an exhibit, a sample being dropped during handling) or a batch audit entry if required. Significant adverse events, or adverse events for which corrective action is needed will require an investigation to be completed (an OQI may also be required) in addition to the specimen notes.

#### 5.1. Incorrect labelling event

Where there are labelling discrepancies on samples delivered to the DNA Analysis Unit from QPS, an investigation by DNA Analysis Unit staff is <u>not</u> required; as these labelling issues are reported back to QPS (by the validation of the "Labelling discrepancy" EXH line during examination) for their investigation. Prior to processing the sample, an Evidence Recovery scientist/senior scientist needs to ensure that the sample within the packaging is



in fact the correct sample (check details on forensic register, contact QPS sample management unit). After QPS have investigated these occurrences, they communicate any additional required action/s back to the DNA Analysis Unit. Any communication received by DNA Analysis Unit from QPS must be put into AUSLAB (e.g. emails scanned to AUSLAB, phone conversations added as a UR Note to the relevant case).

Where labelling discrepancies have occurred during the processing of exhibits, subsamples or DNA tubes within the DNA Analysis Unit, an investigation is required. Labelling discrepancies may occur as a result of incorrect data entry, barcode misprinting or from the application of an incorrect barcode to a tube. The Senior Scientist Evidence Recovery, Senior Scientist Analytical or the Senior Scientist Quality and Projects must be notified of any instances of labelling discrepancies. Investigations into these occurrences will depend on the nature of each event, however strategies and considerations for an investigation into mislabelling should include:

- An examination of the AUSLAB audit trail to determine when the affected samples labels were printed, and the staff member that printed it (information on samples processed simultaneous, or samples processed by a single person can be obtained from AUSLAB extended enquires for the purpose of investigation). Using the information from AUSLAB audit trails, from discussions with staff, and from worksheets or examination notes, it should be possible to determine the number of potential labelling errors that may have occurred. The information may be of use to determine how the mislabelling happened. For example: if a mislabelling occurred during examination, other samples processed by that sampling scientist, or other sample barcodes printed at the same time could potentially be affected.
- A review of the documentation which relates to the processing of the sample is required (e.g. examination notes, analytical worksheets, AUSLAB batch audit entries) to see if the correct identity of the sample can be established.
- A confirmation of sample type should be completed as an identity check, and/or to provide additional information to an investigation. For example if examination notes indicate that sample barcode 123456789 should be associated to a swab, but on retrieving the sample it is noted to contain a cigarette butt, a sample/barcode switch should be investigated.
- An assessment of the AUSLAB tracking of the sample may be informative. In situations where barcode labels have been switched (between two items), mis-printed, or duplicate labels printed, evidence on the time at which this occurred may be obtained from AUSLAB storage records.

### Corrective actions and documentation:

In <u>all</u> cases of mislabelling, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, the teams' events register, and/or in I:\Quality & Projects\Investigations (Refer to <u>section 8.2.3</u>).

If the sample can be positively identified as a result of the investigation, the result may be reported after the completion of corrective actions and documentation as described above. If the sample can <u>not</u> be positively identified, in consultation with the Senior Scientist Quality and Projects or a Team Leader the sample (or sub-sample) may be failed. Where a sample is failed, if it is possible - it should be re-sampled/re-extracted. If it is not possible to positively identify the sample, or to reprocess the sample, the sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS



on sample failures will only occur in consultation with the Senior Scientist Quality and Projects or a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

## 5.2. Sample cross contamination

Sample cross-contamination can occur between exhibits, between DNA samples, or from staff to exhibits/samples. The type of contamination that has occurred will determine how/when the contamination is detected and how it will be investigated. Detection of cross contaminations are usually identified after profiling.

Detection of staff contamination of samples can be identified at plate reading by:

- the <u>staff match macro</u> (for casework and reference samples) which identifies potential matches between samples and DNA Analysis staff, prior to result upload to AUSLAB.
- the <u>AUSLAB staff match function</u> (for casework samples only) which identifies potential matches between samples and QPS staff, after result upload to AUSLAB

Detection of sample-to-sample contaminations can be identified by:

- The extraction batch (EB) check (performed on auto-extraction batches processed on the MPII)
- Case management and reporting processes
- Link creation/confirmation
- Incorrect profile in positive or negative controls

Detection of staff to sample or sample-to-sample contaminations may also be identified from quality searches (as performed by the Quality Team)

Where a cross contamination event is suspected, in consultation with the Senior Scientist Evidence Recovery, Senior Scientist Analytical or Senior Scientist Quality and Projects the following actions should be considered:

- If a possible contamination event of an exhibit/DNA sample by a staff member is identified by the staff match macro or by a quality search, AUSLAB records including audit trails, and/or FBX fields should be reviewed to establish if the staff member has contacted the exhibit/DNA during processing i.e. during examination, DNA extraction etc. If there is no evidence that the staff member has contacted the sample, an analytical investigation is required (Refer to section 8). For environmental monitoring samples which contain a possible staff match: Refer to QIS 23602 for required actions.
- If a contamination between exhibits is suspected, AUSLAB records should be reviewed (by extended enquiry function, user audits and/or audit trails) to establish who has handled the exhibits, when they were processed/moved, and where the exhibits have been located/examined. It may also be useful to refer to Forensic Register records (from QPS). This information should enable any potential cross contamination events due to physical proximity (time/place/staff handling) to be identified. The possibility of transfer of DNA from exhibit to equipment (e.g. tweezers) and equipment on to the next exhibit should also be considered (swabbing and profiling the equipment may assist an investigation). If there is no evidence of physical proximity of the exhibits under investigation, an analytical investigation will be required (Refer to section 8).
- If a contamination between DNA samples is suspected, an analytical investigation is required (Refer to section 8).



# **Corrective actions and documentation:**

In <u>all</u> cases of cross-contamination, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, the teams' events register, and/or in <a href="https://linearcharm.nih.gov/linearcharm.nih.

If after investigation it is determined that the sample/s have not been contaminated the results may be reported after the completion of the investigation documentation as described above. If after the investigation it is determined that the sample has been contaminated, in consultation with the Senior Scientist Quality and Projects or a Team Leader the sample may be failed. When a sample (or sub-sample) is failed, if it is possible - the item should be re-sampled/re-extracted and profiled. If it is not possible to reprocess the item, the item/sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS on sample failures will only occur in consultation with the Senior Scientist Quality and Projects and a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

# 5.3. Incorrect use of reagents

The incorrect use of reagents during the preparation of samples, or in the completion of a presumptive screening test, has the potential to detrimentally impact on further presumptive testing, DNA extraction and/or profiling results. If incorrect reagent usage is suspected, an investigation is required and the Senior Scientist Evidence Recovery, Senior Scientist Analytical or Senior Scientist Quality and Projects should be advised. The investigation into incorrect reagent usage should include:

- A check of the labelling on the reagents used for sample processing. Ensure that the correct reagent has been used, and that the reagent has not expired.
- Review all the reagents that have been used for the processing of the sample as shown in the AUSLAB consumables audit trail. A check of other samples processed with the same reagent/s, is required to determine if the reagent has functioned (as expected) on previously tested samples.
- If the reagent is specific to a presumptive test, repeat the presumptive test with the suspected incorrect reagent (and if possible a known functional reagent) with the presumptive tests positive and negative controls (Refer to section 4). The function of the test on the controls - may provide information on the correct function of the reagents and/or the presumptive test.
- Note any unusual test results or test performance issues
- Ensure that the correct procedure has been used (refer to active QIS document as applicable)

Before any further testing is conducted, reagents should be re-prepared (if applicable), purchased (if applicable), and/or retested with positive and negative controls. All quality controls (positive and negative) must pass the criteria as outlined in <a href="mailto:section 4">section 4</a>, section 8 and/or <a href="mailto:section 9">section 9</a> before further testing can be conducted on casework/reference samples.

# **Corrective actions and documentation:**

In <u>all</u> cases of incorrect reagent usage, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, and/or in I:\Quality & Projects\Investigations (Refer to section 8.2.3).



If after investigation it is determined that the sample/s have not been adversely affected, the results may be reported after the completion of the investigation documentation as described above. If after the investigation is complete it is determined that the sample has been adversely affected by incorrect use of reagents, but still has some evidentiary value, the impact of the event of the sample should be described in specimen notes.

If the sample is no longer suitable for reporting due to the adverse event, in consultation with the Senior Scientist Quality and Projects and a Team Leader the sample may be failed. Where a sample (or sub-sample) is failed, if it is possible - the item should be resampled/re-extracted and profiled. If it is not possible to reprocess the item/sample, the sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS on sample failures will only occur in consultation with the Senior Scientist Quality and Projects and a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

# 6. CASEWORK EXTRACTION AND AMPLIFICATION BATCHES: RESULTS ACCEPTANCE CRITERIA

Microcon and nucleospin batches contain a negative control only. For microcon and nucleospin batches, the negative control must pass (refer to Figure 2) for the batch to pass. If the negative control is <u>not</u> No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before initiating investigation processes.

All other casework extraction and amplification batches contain a minimum of one positive control and one negative control. If the positive control within a batch is <u>not</u> the expected full profile, and/or the negative control is <u>not</u> No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before initiating investigation processes. For an extraction or amplification batch to pass, **both the positive and negative controls must pass** as determined by the quality criteria indicated in Figure 1 (positive control criteria) and Figure 2 (negative control criteria).

In some rare circumstances, where the amplification batch positive control fails, it may be possible to use a sample as a "positive control", if the sample has been previously profiled and the profile results for the sample in this batch match its' previous profile (this approach requires consultation with the Team Leader, Senior Scientist Quality and Projects, or Senior Scientist Analytical).

Where the positive and/or negative control profile is not ideal (i.e. expected full profile for the positive control, and NSD for the negative control) there are specific actions which must be completed, and batch details assessed before the batch can be passed (Refer to Figure 1 and Figure 2). Figure 1 and Figure 2 outline the required actions and batch considerations which must be made prior to passing or failing a batch. The actions and considerations are dependant on the control profile/s results (i.e. partial profile, excess, or a mixture profile). The required actions and batch check details in Figure 1 and Figure 2 are brief, for full details of requirements for each action refer section 8.

#### 7. QUANTIFICATION BATCHES: RESULTS ACCEPTANCE CRITERIA

Quantification batches have several quality criteria which need to be assessed to determine if the batch is passed or failed (Refer to Figure 3). In circumstances where quality criteria/thresholds are not met, the batch requires review and is to be discussed with the Senior Scientist Analytical (or Team Leader/Senior Scientist Quality and Projects) to determine batch outcome.



### Investigating Adverse Events in DNA Analysis Unit

An initial evaluation of the extraction negative controls occurs during the quantification process (refer to section 6, and Figure 2 for additional information on extraction control quality guidelines). The quality control criteria and actions for quantification values in extraction negative controls are also detailed in the Quantification of Extracted DNA (19977) standard operating procedure.



#### Investigating Adverse Events in DNA Analysis Unit

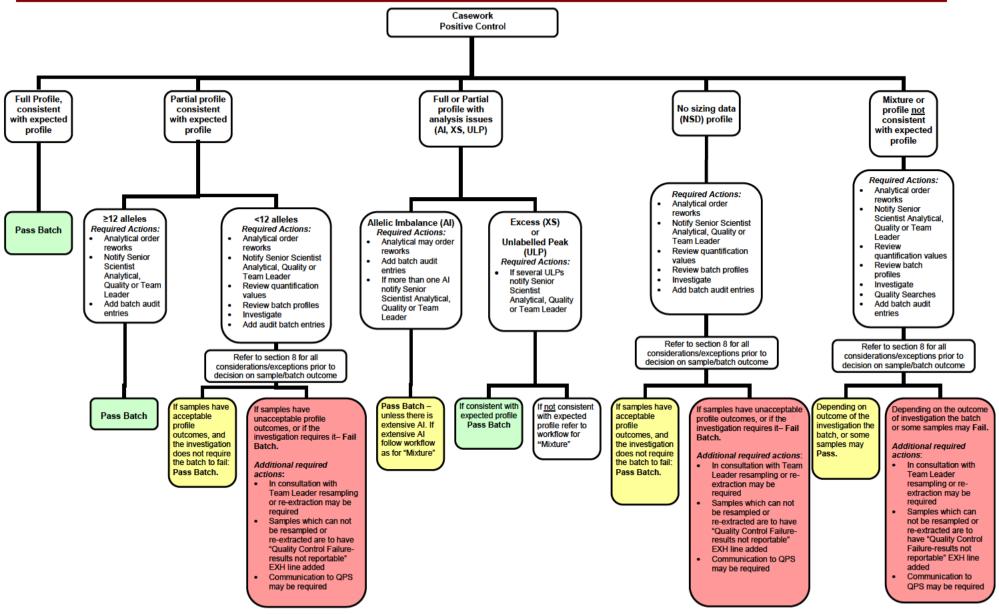


Figure { SEQ Figure \\* ARABIC }: Casework Positive Control Workflow (Extraction and Amplification Batches). Required actions and batch check details are brief - refer to section 8 for full details of requirements.

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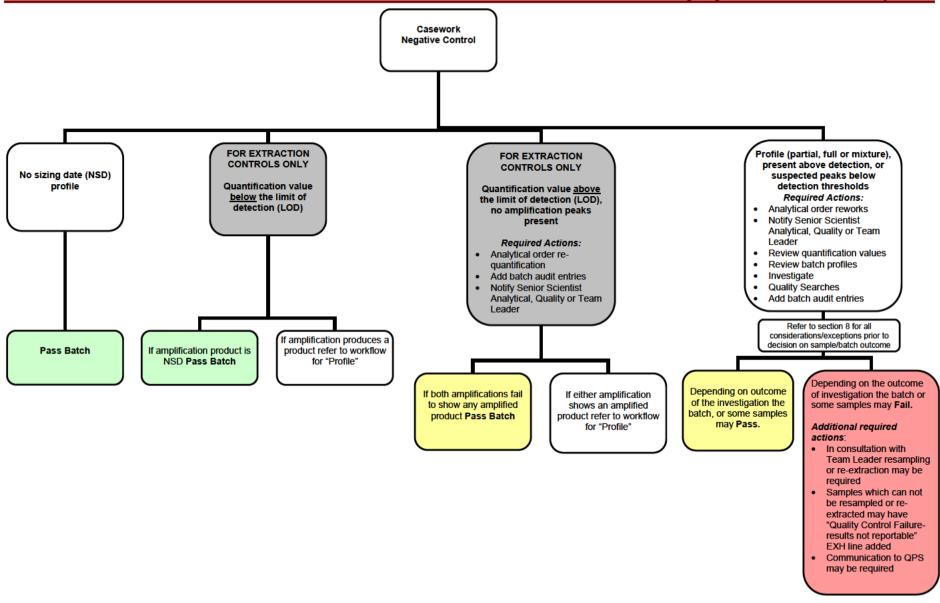


Figure 2: Casework Negative Control Workflow (Extraction and Amplification Batches). Amplification negative controls are not processed through quantification - quantification values do not apply. Required actions and batch check details are brief - refer to section 8 for full details of requirements.

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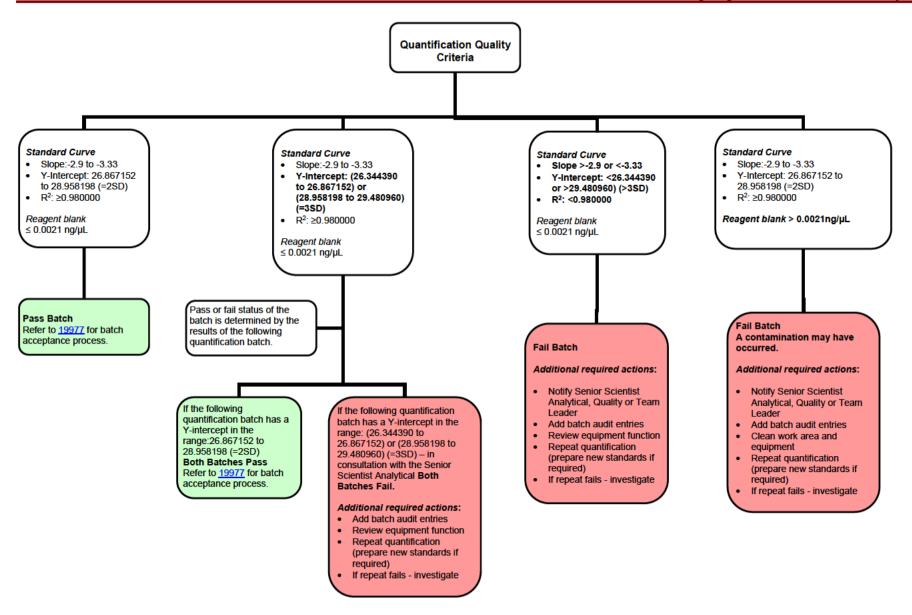


Figure 3: Quantification quality criteria and required actions. Required actions and batch check details are brief - refer to section 8 for full details of requirements.

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# 8. INVESTIGATIONS INTO ADVERSE ANALYTICAL EVENTS: INCLUDING CONTROLS OUTSIDE ACCEPTANCE CRITERIA

In cases where unexpected profile/s are obtained from positive controls, DNA is detected in negative controls (from extraction or amplification batches), or a laboratory processing event has occurred which has the potential to cause a DNA contamination event, an investigation into the adverse event is required. Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Analytical and the Senior Scientist Quality and Projects. Where possible, all results from batches under investigation should be placed on-hold until the outcome of the investigation is complete. Investigations should include the actions as described in sections 8.1-8.7.

# 8.1. Repetition of CE results prior to investigation

If the positive control within a batch does not pass (shown in yellow/red) in Figure 1, and/or the negative control within a batch does not pass (shown in yellow/red) in Figure 2, Analytical staff will re-CE the plate to confirm the profile outcome (i.e. is it reproducible), before an investigation is initiated (also consider CE carry-over as a possible source of unexpected alleles - particularly negative controls). Other adverse events may also require re-CE to confirm the adverse event is reproducible, before an investigation is initiated.

Analytical staff will order reworks on controls as per standard operating procedures QIS 24012 and QIS 17130 where it is indicated as necessary by the workflows in Figure 1 and Figure 2. Analytical staff should consider quantification values of the controls, the quantification values of the samples on the batch and profiling results before ordering the rework/s. Where an investigation is required, additional reworks may be requested by the staff that are completing the investigation (refer to section 8.7)

# 8.2. Investigation process

#### 8.2.1 Results management

For samples or batches under investigation, where it is possible - <u>no</u> results should be released until the investigation deems it suitable to do so (part or all of the results may be released after the investigation – depending on the results). This may require the following actions:

- Where possible do not upload to AUSLAB results until the investigation is complete. If the investigation results in the batch failing – Do not upload failed batch results
- Add potentially affected samples/batches to the QEXH list, and remove them from case management lists.
- "DO NOT USE" may be added to 9PLEX or 9FTAR result (if results have already been uploaded to AUSLAB but not yet reported).
- Adding specimen notes and batch audit entries immediately. Batch audit entry should indicate that an investigation is required. For analytical investigations refer also to QIS 24012 for additional information on specimen and batch notes.
- If results have been reported discuss required actions with Senior Scientist Quality and Projects and the Team Leaders.
- Senior Scientist should email details of the investigation to the Management Team.

### 8.2.2 Required actions and considerations in investigations

Investigations should include the following steps:



- Batch audit entries must be used to detail the investigation process (refer to section 8.3).
- Review of batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected samples or batches that are under investigation.
- Review the controls that relate to the sample/batches under investigation to ensure they
  meet quality criteria as detailed in Figure 1 5 as applicable.
- Review the batch profiles (refer to section 8.5) and quantification values if useful (refer to section 8.4).
- It may be useful in some circumstances, to check the function/programming of the equipment that was used (e.g. was the correct program used on the thermal cycler, was the performance of the 3130xl Genetic Analyzer suitable for interpretation).
- A check of the controls, chemicals/kits and reagents that have been used may be useful (information located on worksheets and AUSLAB material audit history) including:
  - correct control for the batch (e.g. the correct FTA control card punched)
  - expiry date of reagents/kit
  - has the reagent/kit functioned on a previous and a subsequent batch
  - in cases of contamination, consider reagents/chemical as a possible source
- In consultation with Team Leaders and Senior Scientist Quality and Projects order reworks (e.g. microcons, re-amplification, re-extractions etc) if they will provide additional information to the investigation. Refer to section 8.7 for rework strategies for investigation purposes. However before reworks are ordered the amount of sample available for testing should be carefully considered. Additional quality searches and batch checks may be required on reworked samples.
- Complete quality search if applicable (refer <u>section 8.6</u>). There may be instances where a quality search is completed at the beginning of an investigation and then repeated after rework results have been obtained.
- Raising an OQI should be considered, particularly in instances of a significant or reoccurring adverse event. If an OQI has been raised – the findings of the investigation will be recorded within QIS.

# 8.2.3 Documentation of investigation

On completion of the investigation, detailed batch audit entries (refer section 8.3) and/or specimen notes should be completed for all affected samples.

<u>Where results are released</u> to AUSLAB for interpretation (and there have been unexpected processing issues or profiling results for the sample/batch) notes should include: a description of the adverse event, the investigation that was completed, the corrective actions completed (if applicable), the impact of the event on the sample/s, and the considerations that are required for the interpretation of the profile/s as a result of the issue.

Where results are not suitable for release notes should include: a description of the adverse event, the investigation that was completed, the corrective actions completed (if applicable) and the impact of the event on the sample/s. A clear statement that the results are not suitable for interpretation or reporting should be made.

Failed samples/batches will need to be repunched/reworked (if they are reference samples), or reworked/re-extracted/resampled or failed (if they are casework). Failure of samples will occur only in consultation with a Team Leader/Senior Scientist Analytical/Senior Scientist Quality and Projects, and may require additional communications



with the QPS. If needed, supporting data and information for investigations into adverse events can be stored to network drive <a href="L:\Quality & Projects\Investigations">L:\Quality & Projects\Investigations</a> or the the teams' events register. Issue/s and findings (including OQIs) may also be discussed in relevant team meetings, to alert staff to quality issue/s.

#### 8.3. Batch audit entries

Batch audit entries must:

- Be entered in a timely way.
- Should be added progressively if the batch is under investigation. For example if a batch is on hold pending the results of an investigation, the batch audit entry must state that the batch is under investigation. As reworks are ordered for the purpose of investigation, the details of the reworks and the implications of the findings of the rework/s should be stated in the batch audit entry.
- For analytical investigations refer to QIS 24012 for additional information on specimen and batch notes.
- Clearly state if the batch fails, passes (with no quality issues) or passes (but has been affected by one or more quality issues which are detailed in the batch audit entries)
- If there is a quality issue with the batch the batch audit entry must clearly state what the issue is, the action/s taken (i.e. investigation details), and the outcome of the actions/investigations.
- If there is a quality issue with the batch the batch audit entry must be accompanied by specimen notes on all samples on the batch; the specimen note must refer to the batch audit entry e.g. the specimen note would state: "Refer to batch audit entry CWGMP2012XXXXXX\_XX, in addition to having "See specimen note" entered into the comment field for each 9PLEX or 9FTAR page (as applicable).
- Where the negative control on a batch has a quantification value, the batch audit entry must state the quantification value obtained from the negative control, and state if that value is >or< the limit of detection (LOD), or limit of reporting (LOR).</p>
- If an OQI is raised as a result of findings/investigations, the batch audit entry, and specimen notes (for all samples on the batch) should have the OQI number entered into the notes.

### 8.4. Review quantification values (controls and samples)

Reviews of quantification values of individual samples and/or a batch is beneficial when:

- a quantification batch is under investigation due to the controls not meeting the quality criteria as described in Figure 3
- a negative control has a quantification value (particularly >LOD)
- an adverse event has occurred that impacts significantly on DNA yield
- an adverse event has occurred and the quantity of DNA in the samples adversely affected would inform the investigation.
- to determine if reworks should be ordered for investigation purposes

A review of a quantification batch, requires a scientist to make an assessment of the expected quantification values (based on sample type and previous quantification results) in comparison with the quantification values obtained from the sample/batch under investigation.



# 8.5. Review batch profiles (controls and samples)

Where an unexpected profile has been obtained in a control, or there has been an adverse event on a batch which has required investigation, a check of the profiles from the other samples and controls in the batch is required. This check is usually completed in Genemapper ID-X (not Auslab), so that below reporting threshold peaks can be reviewed/assessed. All controls that related to a batch and/or samples under investigation should be reviewed to ensure they meet acceptable quality criteria (refer to Figure 1-5 as applicable).

<u>For casework</u> samples that have been processed on an automated platform this batch check may include an Extraction Batch (EB) check at lowered thresholds (refer to QIS 17119).

<u>For FTA reference</u> batches - the batch review should include a visual inspection of the plate to ensure the correct location and number of spots is present in each well (Refer to section 11 for FTA investigations).

The purpose of the batch review is to:

- Identify any additional quality issues on the batch/plate (if present)
- Establish possible sources of unexpected alleles within a control/sample that
  may have sourced from within the batch/es in which the sample/controls have
  been processed (if applicable).
- To assess if an event has impacted on some or all of the controls/samples (e.g. poor amplification)

Examples of batch reviews:

- Where a negative control contains a part or full profile, the review of the batch would aim to determine if any samples from within that batch could have contributed to the alleles that have been observed in the negative control.
- If a positive amplification control was NSD the batch check would determine if it is the control only, or the entire batch that failed to amplify.
- If an FTA or FTA control produced a mixture profile, the batch review would be searching for the source of the additional alleles from FTAs processed on the same batch/plate.

Instances where adverse events impact on casework samples are more difficult to investigate, and may require mixture interpretation to determine if cross-contamination within batches has occurred.

# 8.6. Quality searches

Quality searches are to be performed when the source of an unexpected profile is not able to be determined (e.g. a profile in a negative control that does not match a sample on a batch). Quality searches can <u>only</u> be completed by the Managing Scientist or the Senior Scientist Quality and Projects. If a quality search is required, a copy of the profile requiring a search will be required. A quality search consists of a search against DNA Analysis staff, QPS staff (if applicable), the unknown profiles database, and a search against all casework and all reference samples that have been processed within DNA Analysis. The quality search may identify possible sources of the unknown profile, and can inform investigations.

In cases where the source of an unknown profile involved in an investigation is not able to be determined, the unknown profile will be uploaded to the "Unknown profile" database that is



maintained by the quality team. This will ensure that any future occurrences of this profile can be identified.

# 8.7. Rework strategies for investigation purposes

Reworks including microcons, nucleospins or re-amplifications should be requested if they will provide additional information to the investigation. However <u>before reworks</u> are ordered the amount of sample available for testing should be carefully considered. Examples of the use of reworks for investigations include:

## Improving profiles for quality searches/match purposes:

- A microcon may be ordered to increase the number of alleles present in a partial/below threshold profile
- Reamplifications at higher DNA concentrations to increase available alleles

# Reworks to establish time/source of contamination/s:

- A re-preparation/CE may establish if a contamination occurred at/prior to amplification (if the result is reproducible) or occurred during CE (if the result is not reproducible)
- A re-amplification may establish if a contamination occurred at/prior to extraction (if result is reproducible) or occurred during amplification (if result is not reproducible)
- A re-extraction/re-punch may establish if a contamination occurred during extraction

The quantification values for samples under investigation should be considered. Samples with low quantification values may not produce uniform profiling results - due to the stochastic effect of PCR. Samples with high quantification values should profile consistently. Additional quality searches and batch checks may be required on reworked samples.

# 9. FTA REFERENCE BATCH CONTROLS: RESULTS ACCEPTANCE CRITERIA

FTA reference batches (this does <u>not</u> include EFTA batches – Refer to section 10 for EFTA samples) contain two positive controls (1 spot control, 2 spot control) and a negative control. If the positive control/s within a batch are <u>not</u> the expected full profile, and/or the negative control is <u>not</u> No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before investigation processes are initiated. For an FTA batch to pass, **both a positive and negative control must pass** as determined by the quality criteria indicated in Figure 4 (positive control criteria) and Figure 5 (negative control criteria). Given that each FTA batch contains two positive controls, the best of the two control profiles is assessed in the Figure 4 workflow to determine required actions for the batch (i.e. if one of the positive controls passes the batch can be accepted, even if the second positive control may not meet required quality criteria).

In some rare circumstances, where the batch positive control fails, it may be possible to use a sample as a "positive control", if the sample has been previously profiled and the profile results for the sample in this batch match its' previous profile (this approach requires consultation with the Team Leader and Senior Scientist Quality and Projects).

Where the positive and/or negative control profile is not ideal (i.e. expected full profile for the positive control, and NSD for the negative control) there are specific actions which must be completed, and batch details assessed before the batch can be passed (Refer to Figure 4 and Figure 5). Figure 4 and Figure 5 outline the required actions and batch considerations which must be made prior to passing or failing a batch. The actions and considerations are dependant on the control profile/s results (i.e. partial profile, excess, or a mixture profile). The required actions and batch check details in Figure 4 and Figure 5 are brief, for full details of requirements for each action refer section 8 and section 11.



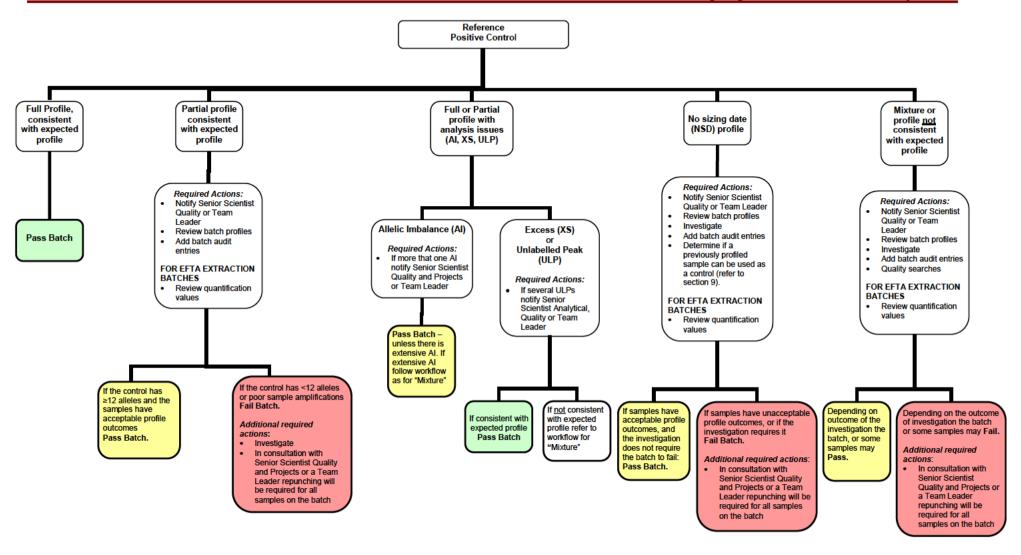


Figure 4: Reference Positive Control Workflow (EFTA Extraction, EFTA Amplification and FTA Batches). FTA batches have two positive controls, the best of the two control profiles is assessed in this workflow to determine required actions. Refer to section 8 for full details of required actions.

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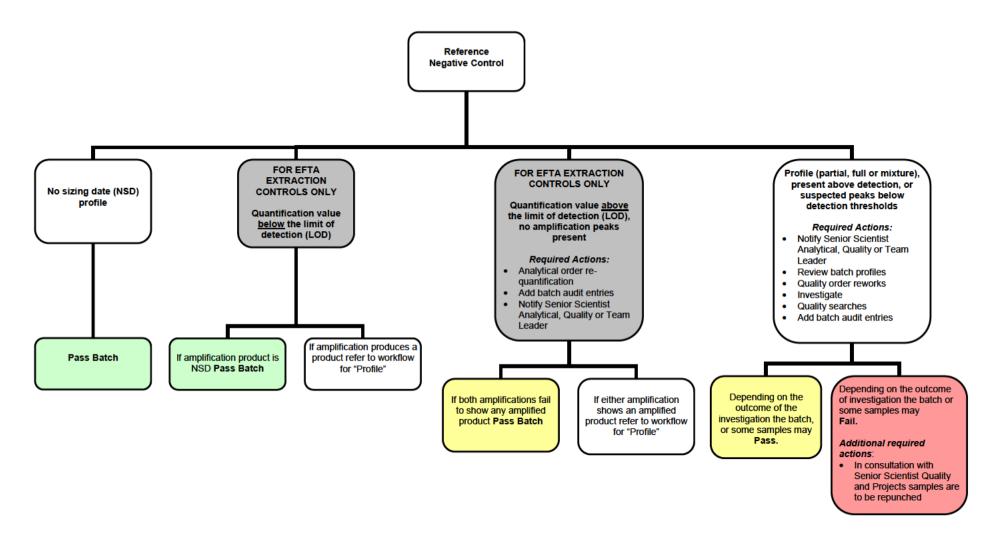


Figure 5: Reference Negative Control Workflow (EFTA Extraction, EFTA Amplification and FTA Batches). Amplification negative controls are not processed through quantification - quantification values do not apply. Required actions and batch check details are brief - refer to section 8 for full details of requirements.

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# 10. EXTRACTION FTA (EFTA) BATCH CONTROLS: RESULTS ACCEPTANCE CRITERIA

FTA samples which are processed through a DNA IQ extraction process - are Extraction FTA samples (EFTA). EFTA punch batches contain a negative control only, and do not contain a positive control on the batch. EFTA differ from standard FTA processing, as the samples are processed through an extraction batch, a quantification batch and an amplification batch - each of which have specific controls. EFTA sample quality guidelines and required actions for each step of processing are listed in Table 1. In circumstances where an EFTA batch fails (as a result of not meeting the quality criteria) adequate batch audit entries and specimen notes are required, and an investigation should be initiated (Refer section 8 and section 11). Where a significant or reoccurring quality issue is identified an OQI should be raised.

Batch Type	Control	Actions
Punching batch	Negative Control	EFTA negative control pass/fail batch status and required actions are the same as those for standard FTA Negative Controls Refer to Figure 5 for workflow
Extraction	Positive Control	EFTA positive control pass/fail batch status and required actions are the same as those for standard FTA Positive Controls Refer to Figure 4 for workflow
Batch	Negative Control	EFTA negative control pass/fail batch status and required actions are the same as those for standard FTA Negative Controls <b>Refer to Figure 5</b> for workflow
Quantification Batch		EFTA batch control pass/fail batch status and required actions – Refer to <b>Figure 3</b> for workflow.
Amplification	Positive Control	EFTA positive control pass/fail batch status and required actions are the same as those for standard FTA Positive Controls Refer to <b>Figure 4</b> for workflow

Table 1: Extraction FTA (EFTA) batches: quality criteria and required actions.

#### 11. INVESTIGATION PROCESSES FOR ADVERSE EVENTS IN FTA PROCESSING

Refer to Figure 5 for workflow

In cases where a mixed profile results from an FTA sample, an unexpected profile is obtained from a positive control, DNA is detected in a negative control, or a laboratory processing event has occurred which has the potential to cause a DNA contamination an investigation into the adverse event is required. In each case, the adversely affected control/sample should undergo a re-CE to confirm the adverse event is reproducible, before an investigation is initiated.

EFTA negative control pass/fail batch status and required actions

are the same as those for standard FTA Negative Controls

Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Quality and Projects or Team Leader. Where possible, all results from batches under review should be placed on-hold until the outcome of the investigation is complete.

<u>For EFTA batches:</u> If an EFTA extraction, quantification or amplification batch fails – only the samples on the affected batch/es will be failed. These failed samples will likely be distributed over several plate reading batches. For failed EFTA batches partial plate



Batch

Negative

Control

reading batches may thus need to be uploaded (i.e. with the failed samples removed). This differs from FTA plates which are processed together during punching, amplification and plate reading.

Investigations should include the following actions:

- Ensure results are not incorrectly utilised or reported. Refer to Section 8.2.1 for results management guidelines
- Check all positive and negative control samples meet quality guidelines (Refer to Figure 4 and Figure 5).
- Review batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected samples or batches that are under investigation.
- For FTA batches visually inspect the plate (not applicable to EFTA batches) for the correct number of punch spots in each well.
- Batch profiles must be checked refer to <u>section 8.5</u>. For EFTA batches a review of quantification results may also be required (refer to section 8.4).
- It may be useful in some circumstances, to check the function and or programming of the equipment that was used (e.g. was the correct program used on the thermal cycler, was the performance of the 3130xl Genetic Analyzer suitable for interpretation).
- A check of the controls, chemicals/kits and reagents that have been used may be useful (information located on worksheets and AUSLAB material audit history) including:
  - correct control for the batch (e.g. the correct FTA control card punched)
  - expiry date of reagents/kit
  - has the reagent/kit functioned on a previous and subsequent batch
  - in cases of contamination, consider reagents/chemical as a possible source
- For EFTA investigations order reworks (e.g. microcons, re-amplification etc.) if they will provide additional information to the investigation. Refer to section 8.7 for rework strategies for investigation purposes. Additional quality searches and batch checks may be required on reworked samples.
- Complete quality search if applicable (refer section 8.6). There may be instances where a quality search is completed at the beginning of an investigation and then repeated after rework results have been obtained.
- Raising an OQI should be considered, particularly in instances of a significant or reoccurring adverse event. If an OQI has been raised – the findings of the investigation will be recorded within QIS.
- All investigation findings must be documented as per section 8.2.3
- A photocopy of the failed batch/plate paperwork to be given to Quality Team, as it will be filed in the "FTA Investigations" folders.
- On completion of the investigation: ensure all affected reference samples have been reprocessed, such that reportable results are available.

#### 12. INVESTIGATION INTO REFERENCE SAMPLE MIXTURE PROFILES

Reference samples are expected to be single source samples. In cases where a mixed profile is obtained from an FTA sample, an investigation is required. The investigation will aim to determine if a DNA contamination has occurred within the DNA Analysis Unit



Laboratory or if the sample that was submitted to DNA Analysis (as a reference sample) was not a single source specimen.

A mixture in a reference sample may result from occurrences such as: an FTA card contamination (pre or post delivery to DNA Analysis), BSD punch carryover, FTA spots moving within a plate, labware contamination, reagent contamination, cross contamination during washing/extraction/quantification/amplification, or in very rare circumstances it may be the correct profile for a person.

Before an investigation is initiated the adversely affected sample should under go a re-CE to confirm the adverse event is reproducible. Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Quality and Projects. Where possible, all results from batches under review should be placed on-hold until the outcome of the investigation is complete.

If the re-CE confirms that the result is reproducible, the following **initial investigation steps are required:** 

- Review batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected the sample.
- For FTA batches visually inspect the plate (not applicable to EFTA batches) for the correct number of punch spots in each well.
- Batch profiles must be checked refer to section 8.5.
- Check all positive and negative control samples meet quality guidelines (Refer to Figure 4 and Figure 5).
- Complete quality search (refer <u>section 8.6</u>).

# 12.1. Investigation actions for FTA samples with a reproducible mixture

If on completion of the initial investigation actions above (section 12) it is determined that there are multiple quality issues with the plate (i.e. multiple samples on the plate contain mixtures, control failures) the plate should be **failed**. For a failed batch refer to <u>section 11</u> for investigation processes and required actions.

If there is only one sample on the plate/batch that is a mixture, but the source of the mixture is not able to be determined, after completion of the initial investigations actions above (section 12), a REPUNCH of the sample which has produced the mixture profile should be requested. The plate/batch on which the mixture sample was processed should be placed on hold pending the results of the RPUNCH rework.

If the <u>REPUNCH</u> of the sample confirms the mixture profile, and there are no additional mixtures or analysis issues identified during the batch profiles check (refer to <u>section 8.5</u>), in consultation with Senior Scientist Quality and Projects - the batch may be **passed**. All investigation findings must be documented as per section 8.2.3. The batch can be passed as the mixture has been confirmed as the correct profile for that FTA card, and not as a result of a sample processing issue. However, due to the FTA card producing a mixture profile, it is not suitable as a reference sample and as such a Team Leader should also be advised as a new FTA sample needs to be requested from QPS.

If the <u>REPUNCH is single source</u> in consultation with Senior Scientist Quality and Projects the batch - should be **failed**, as a contamination event has occurred. For a failed batch refer to section <u>section 11</u> for investigation processes and required actions.



#### 12.2. Investigation actions for EFTA samples with a reproducible mixture

If a mixture profile in an EFTA sample is reproducible (after re-CE) but the initial investigation actions above (section 12) are not able to determine the source/cause of the mixture profile the following actions are required:

Order a re-extraction (EFTA) of the mixture FTA samples (under a connected barcode) and a re-amplification from the initial EFTA sample to determine if the contamination has occurred prior to extraction, during extraction or during amplification. Where possible the batches on which the mixture sample was processed should be placed on hold pending the rework results.

If the <u>re-extraction and re-amplification confirm the mixture profile</u> (and there are no additional quality issues identified during the initial investigation actions above (<u>section 12</u>), in consultation with Senior Scientist Quality and Projects - the batch may be **passed**. All investigation findings must be documented as per section 8.2.3. A Team Leader should also be advised as a new FTA sample may be required.

If the <u>re-extraction and re-amplification is single source</u> (or there are quality issues with the batch/es) in consultation with Senior Scientist Quality and Projects - the batch should be **failed**. For a failed batch refer to <u>section 11</u> for investigation processes and required actions. The results from the re-extraction and re-amplification will provide information to the investigation on the likely time/process at which the contamination occurred.

If an EFTA extraction, quantification or amplification batch fails – <u>only</u> the samples on the affected batch/es will be failed. These failed samples will likely be distributed over several plate reading batches. For failed EFTA batches partial plate reading batches may thus need to be uploaded (i.e. with the failed samples removed).

# 13. INVESTIGATING A SAMPLE WHICH HAS DIFFERENT PROFILING RESULTS

Where a casework or a reference sample is profiled twice, and the two resulting profiles do not match, Re-CE of both amplified profiles (Refer to QIS 17130) should be ordered (Note: alleles present in a mixture may vary between amplifications). After Re-CE:

If it is confirmed that both sample profiles are the same:

- Advise Senior Scientist Quality and Projects
- Investigate incorrect CE result (refer to <u>section 8</u> for casework, section 11 for reference samples)
- Ensure that no incorrect profiles have been reported.

If it is confirmed that the sample profiles are different:

- Advise Senior Scientist Quality and Projects
- Order re-extraction (casework) or re-punch (reference) of the sample.
- Investigate incorrect CE result (refer to <u>section 8</u> for casework, section 11 for reference samples)
- View samples to ensure they have been correctly labelled
- Ensure that no incorrect profiles have been reported.

All investigation processes, actions and reporting in relation to this type of adverse event will be as described <u>section 8</u> for casework, section 11 for reference samples.



#### 14. RECORDS

AUSLAB batch audit entries, specimen notes and UR notes (as appropriate) will detail results from adverse events, adverse event investigation/s and outcomes of investigations. OQIs within QIS may used: particularly in instances of a significant adverse event. If needed, supporting data and information for investigations into adverse events can be stored to network drive I:\Quality & Projects\Investigations.

Where investigations into reference batch failures have been completed a photocopy of the plate/batch paperwork should be given to Quality to be filed in the "FTA Investigations" folders.

#### 15. ASSOCIATED DOCUMENTATION

QIS: 10001 Quality Information System  OIS: 12005 Opportunities for Quality Improvement (QQIs) Management Presedure (QQSS)
QIS: <u>13965</u> Opportunities for Quality Improvement (OQIs) Management Procedure (CaSS) QIS: 17119 Procedure for the Release of Results
QIS: <u>17130</u> CE Quality Check of Samples from the ABI Prism 3130xl Genetic Analyzers
QIS: 17154 Procedure for Quality Practice in DNA Analysis
QIS: <u>17155</u> Procedure for Errors, Major Concerns, System Breakdowns
QIS: 17186 The acid phosphatise screening test for seminal stains
QIS: <u>17190</u> Tetramethylbenzidine screening test for blood
QIS: 17193 Phadebas test for saliva
QIS: 19976 Amplification of Extracted DNA using the AmpF{STR® Profiler Plus® Kit or AmpF{STR® COfiler® Kit
QIS: 19977 Quantification of Extracted DNA using the Quantifiler™ Human DNA Quantification Kit
QIS: 24015 Procedure for Intelligence Reports and Interstate/Interpol Requests
QIS: 24012 Miscellaneous Analytical Section tasks
QIS: <u>24823</u> FTA Processing and Work Instructions.

# 16. REFERENCES

ABAcard<sub>®</sub> p30 Test For The Forensic Identification of Semen. Technical Information sheet. Abacus Diagnostics, Inc.

#### 17. AMENDMENT HISTORY

Version	Date	Updated By	Amendments
1	5 April 2012	K Scott	First Issue

## 18. APPENDICES

Nil



From: Paul Csoban

Sent: Monday, 12 December 2016 2:49 PM

**To:** Cathie Allen; Jade Franklin

Subject: RE: Project #181

# l agree Paul

From: Cathie Allen

Sent: Monday, 12 December 2016 2:48 PM

To: Jade Franklin Cc: Paul Csoban

Subject: RE: Project #181

#### Hi Jade

I have no issue with it being shared with Mark Brady. My assumption is that Allan would have probably alluded to these documents and the process he undertook in his interview with Mark.

# Cheers Cathie



### **Cathie Allen**

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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From: Jade Franklin

Sent: Monday, 12 December 2016 2:44 PM

To: Cathie Allen Cc: Paul Csoban

Subject: RE: Project #181

Are you both comfortable if I shared this with Mark Brady?

Jade



#### Jade Franklin

Manager Human Resources and Business Relationships People Performance and Excellence Health Support Queensland

From: Cathie Allen

Sent: Monday, 12 December 2016 2:31 PM

**To:** Jade Franklin **Cc:** Paul Csoban

Subject: RE: Project #181

#### Hi Jade

The potential issue that was raised was – it appears that there is a difference between the numbers of sperm seen at Evidence Recovery stage versus the number of sperm seen once Analytical processes had been undertaken – and is this difference of significance. I believe that this issue was raised in early May 2016.

This was investigated by Allan McNevin, with Dr Kirsten Scott as his line manager overseeing the investigation. Allan provided an update to the Management Team about the initial findings of the investigation on the 27<sup>th</sup> of May 2016. Attached are the management meeting minutes and the presentation that Allan provided (excel spreadsheet titled '2016 – Diff Lysis slide micro v original micro).

After the meeting on the 27<sup>th</sup> of May, Allan went on to draft an initial request and then a draft project plan. The initial request is attached titled 'Initial Request #181' and only required approval from the line manager to proceed to a full project plan.

The project proposal and plan were sent to management team on the 1<sup>st</sup> of Sept 2016, with a request for feedback. All feedback sent via email was incorporated into a spreadsheet (titled 'Project#181 Feedback matrix v0.1') so that it was transparent to all management team members the feedback that was put forward and the outcome of the discussion between Emma Caunt and Allan McNevin regarding the feedback and whether it was included in an updated proposal or not (the 2 project staff members. Emma is a HP4 court reporting scientist). I believe that between the first draft and the final signed off proposal, Paula had input into directing the project. I was on leave from the beginning of June until the 6<sup>th</sup> of Sept 2016.

The progress of Project #181 is provided to all management team members at the Management Team meeting (projects are a standing agenda item). Attached is the latest minutes to show that an update was provided.

During the period of this project, Dr Kirsten Scott was acting in the Team Leader role (and therefore Allan's line manager) from 11<sup>th</sup> of April until the 12<sup>th</sup> of June. From the 13<sup>th</sup> of June, Luke Ryan was acting in the Team Leader role until the 12<sup>th</sup> of July when Paula Brisotto returned from Maternity leave to her substantive position.

The substantive management team members are: myself, Wendy Harmer (AO4), Justin Howes (HP6), Paula Brisotto (HP6), Allan McNevin (HP5), Luke Ryan (HP5), Kirsten Scott (HP5), Amanda Reeves (HP5), Kylie Rika (HP5) and Sharon Johnstone (HP5). Only Justin and Paula have been interviewed so far and the interviews were limited to the poor interaction between Allan and Amanda I believe. My assumption is that they will need to be interviewed again to ask about their opinion on the poor working relationship within management team.

Cheers Cathie

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health

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From: Jade Franklin

Sent: Friday, 9 December 2016 4:14 PM

To: Cathie Allen

Subject: Fw: Project #181

#### Cathie

Could you send (what you can) through to me in prep for next year.

#### Jade

Sent from my BlackBerry 10 smartphone on the Telstra Mobile network.

From: Mark Brady

Sent: Friday, 9 December 2016 14:02

To: Jade Franklin Subject: Project #181

# Hi Jade

So I can hit the ground running in the new year, can you get me the documentation for project #181?

# Cheers

Mark Brady Principal Consultant



Employment & Industrial Relations Human Resources & Relationships

Organisational Advisors & Psychologists



Is your leadership making a difference? Based on our award winning leadership program, our new Trusted Leader App is now free to download on the Apple App store and Google Play.

This e-mail, and any files transmitted with it, is intended for the addressee only and may contain confidential information. If you are not the addressee, you are notified that any transmission, distribution or print production of this e-mail and its inclusions is not permitted. The confidentiality attached to this e-mail is not waived, lost or destroyed by reasons of a mistaken delivery to you. If you have received this e-mail and you are not the addressee, please notify us immediately by telephone. Thank you.

From: Cathie Allen

**Sent:** Mon, 12 Dec 2016 10:12:29 +1100

To: Paul Csoban

Subject: Current situation

Importance: High

#### Hi Paul

I've considered the situation with Amanda over the weekend and I have a few questions for Jade and yourself:

- Given Amanda has raised the topic of PID with her lawyer should we advise QH Ethical Standards of this situation? Given that Amanda has discussed internal processes that relate to criminal work with someone from outside the organisation.
- If the answer is Yes to the above, then I would recommend that QPS Ethical Standards is also advised.
- Should we advise the Superintendent of Forensic Services Group of this current situation, given Amanda has engaged a lawyer and discussed information that affects the QPS?
- As FSS has now engaged a lawyer to act on behalf of Forensic DNA Analysis, should her line manager Justin Howes discuss anything with her or should this all now be referred to the QH lawyer? I believe that Amanda has been liaising with Justin and Terry regarding her potential workcover claim. I would appreciate some direction from Jade or yourself on this to ensure that we are following the appropriate procedure.

# Cheers Cathie



#### Cathie Allen

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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From: Cathie Allen

Sent: Friday, 16 December 2016 3:22 PM

To: Jade Franklin
Cc: Paul Csoban
Subject: Summary

#### Hi Jade & Paul

My summary of viewing the information provided:

- It appears that the issue is the same Amanda doesn't believe that enough action has been taken with the incident with Allan and that the perceived issue with respect to examination of slides has been investigated
- Documentation that has been provided includes Management Team Meeting Minutes and a Standard
   Operating Procedure neither of these document types have been approved for release to a third party
- One email shows that Amanda is being disrespectful to another management team member against the Code of Conduct
- Documents AR1, AR2 (i), AR4, AR8, AR11, AR12 and AR13 appear to be missing
- The initial apology from Allan to Amanda has not been included which would also show that she was disrespectful to Allan – which has never been addressed
- It appears that Amanda herself has printed the emails (so assumption is that she's printed the minutes & SOP), so when did that occur? Is that what she had prepared for her meeting with the external investigator? Either way still indicates that she had kept this material at her home.
- Given the release of documents to the lawyer, does this mean this should be referred to Ethical Standards?

In my catch-up with Justin yesterday, he advised that a couple of weeks ago Amanda's handbag was stolen (which included her FSS ID). This incident has upset Amanda greatly – which causes me to question whether this documentation was in her handbag. Amanda's handbag was returned to her – however the money and mobile phone were missing from it (but her ID was in the bag). I'm not sure if this additional information is relevant.

# Cheers Cathie



#### Cathie Allen

Managing Scientist - Police Services Stream

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# **Brief for Approval**

Department RecFind No:	
Division/Business Group:	
File Ref No:	

SUBJECT: Approval to engage Clayton Utz to provide legal services related to Forensic and Scientific Services (FSS) employee Amanda Reeves.

	and Scientific Services (FSS) employee Amanda R	eeves.		
Re	commendation/s			
1.	It is recommended that the Chief Forensic Pathologist FSS: <b>Approve</b> expenditure of up to \$66,000 (Inc. GST) to engage Clalegal services related to FSS employee Ms. Reeves.	ayton Ui	tz to p	rovide
	APPROVED / NOT APPROVED			
	Dr Charles Naylor Chief Forensic Pathologist Forensic & Scientific Services	Dat	e: /	/2016
2.	It is recommended that the Executive Director, Forensic and Scient <b>Approve</b> the engagement of Clayton Utz to provide legal service FSS employee Ms. Reeves exercising Type 2 procurement delegations.	es for th		
	APPROVED / NOT APPROVED			
	Paul Csoban Executive Director Forensic and Scientific Services	Date:	I	/2016
	It is recommended that the Executive Director, People Performan	ce and l	Excell	ence,
	Q: Exercise Type 5 procurement delegation to sign the attached Quo Utz to provide legal services for the case of FSS employee Ms. R			
	APPROVED / NOT APPROVED			
	Dianne Woolley Executive Director People, Performance and Excellence	Date:	I	/2016

Delegate's comments		

Department RecFind No:	
Division/Business Group:	
File Ref No:	

# **Background**

- Ms. Reeves is a long term employee, currently employed as a Senior Scientist in Forensic DNA Analysis.
- 2. Earlier in the year, an issue has been raised regarding the processing of Sexual Assault Investigation Kits and whether spermatozoa are being identified by the current standard operating procedure.
- 3. Identification of spermatozoa during the evidence recovery phase of examination means that an appropriate DNA extraction technique is chosen. If the appropriate DNA extraction technique is not chosen, there is a potential for biological evidence to be lost.
- 4. Additional measures have been put in place to ensure that evidence is not lost and any risks are mitigated, whilst an investigation is conducted into this standard operating procedure to ensure that best practice methods are being used.
- 5. In 2005, an Opportunity for Quality Improvement document (OQI) was provided to the media and resulted in adverse media attention and a Ministerial Taskforce Review.

#### Issue/s

- 6. Ms Reeves initially raised the issue regarding identification of spermatozoa at the evidence recovery phase of examination and this was discussed at Forensic DNA Analysis Management Team meetings.
- 7. On the 9<sup>th</sup> of June 2016, when this issue was discussed at a Forensic DNA Analysis Management Team meeting, Mr Allan McNevin spoke inappropriately to Ms Reeves.
- 8. Ms. Reeves has lodged a number of allegations regarding Mr Nevin with his line manager, Ms Paula Brisotto, regarding the handling of this issue and other issues.
- 9. An external investigation, led by Mr Mark Brady of Livingstones, has commenced to review the poor interaction between Ms Reeves and Mr McNevin, the allegations put forward by Ms Reeves and the poor working relationship between the substantial members of the Forensic DNA Analysis Management Team.
- 10. Mr Brady has completed an interview with Ms Reeves.
- 11. Ms Reeves has lodged a Work Cover Claim and engaged a lawyer regarding this issue.
- 12. In discussions between Ms Reeves' lawyer and Mr Jade Franklin, the lawyer has indicated that his client believes there may be a need for a Public Interest Disclosure (PID) in relation to the identification of spermatozoa during the evidence recovery phase of examination.
- 13. Whilst an internal investigation is being undertaken by the work unit to address this issue, Mr Paul Csoban, Executive Director FSS, will make contact with the Institute of Environmental Science and Research Ltd (ESR) to undertake an external review of this issue. ESR have an excellent reputation in the forensic arena and have previously undertaken an external review for Forensic DNA Analysis in 2005.
- 14. HSQ requires specialised employment law expertise to resolve this matter in light of the complexities around the possible damage to Queensland Health's reputation if Ms Reeves goes forward with a PID, the documents that may be held by Ms Reeves with respect to a PID, and the culmination of the external investigation led by Mr Brady and any issues that may arise from further interviews with team members from Forensic DNA Analysis.

# Vision

15. This brief for approval aligns with the direction set out in the 10-year vision, My Health, Queensland Future: Advancing Health 2026 as follows:

8.1 Delivering Healthcare: Strategic Agenda 2.2 - Empowering our People.

Department RecFind No:	
Division/Business Group:	
File Ref No:	

#### **Results of Consultation**

- 16. Consultation has occurred with the following staff:
  - 1. Paul Csoban, Executive Director, FSS.
  - 2. Cathie Allen, Managing Scientist, Police Services Stream
  - 3. Jade Franklin, HR, HSQ

# **Resource Implications (including Financial)**

17. Funding will be sourced through FSS Operational Budget (787103).

# Attachments

18. Quote from Clayton Utz for legal services provided for Amanda Reeves

Author:	Cleared by:	Content Verified by:
Cathie Allen	Paul Csoban	Jade Franklin
Managing Scientist, Police Services Stream	Executive Director	Manager Human Resource and Business Relationships
Forensic and Scientific Services	Forensic and Scientific Services	People Performance & Excellence

From: Cathie Allen

Sent: Tuesday, 20 December 2016 2:04 PM

To:Paul Csoban; Jade FranklinSubject:Brief for Clayton UtzAttachments:Brief - Clayton Utz.docx

Importance: High

Hi Paul & Jade

I've put the Brief together as best I can.

Cheers Cathie



#### **Cathie Allen**

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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Together Union Meeting	Date: 10.01.2017 Page:
Angelina, Micia, Helen, Tim, S Jade, Paul, CAA, JAH.	san, Heather Hayes
Anom request for A of day for	Evidence
Some notes written or	doc I put together.
Jade Franklin 16.01.20 - Bet HEI applications - directly applications - directly applications in line with policy letters for staff re: 9th signed by S. Kelly & will to me today. Attendees interactions, others is phases - Phase I - autendees, any one else deemed ESE probably need to review to that they consider all as	to send to staff  Tunc - have been  be hard delivered  - letter discusses  re: #181 Will do it in  Phase 2: ER & RI Phase 3-  N ADR'S material.  neuros pet forward.
	. 513

From: Cathie Allen

**Sent:** Wed, 1 Feb 2017 18:18:02 +1100

To: Paul Csoban

**Subject:** ToR for Scientific Review

Attachments: Scientific Review ToR\_20170131.dot

#### Hi Paul

I've put together a small Terms of Reference document for the scientific review in Forensic DNA Analysis. Could you please peer review the document and provide me with feedback.

# Cheers Cathie

# 7

#### **Cathie Allen**

Managing Scientist - Police Services Stream

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# Scientific Review Forensic DNA Analysis

31 January 2017



# **Background**

An independent review regarding evidence recovery processing with respect to sexual assault investigation kits (SAIKs) is requested of the Institute of Environmental Science and Research Limited (ESR), a Crown Research Institute in New Zealand.

An issue has been raised specifically regarding spermatozoa negative, acid phosphatase negative sexual assault samples, however a review of the processing of SAIKs would be appreciated in the spirit of continuing quality improvement.

# **Terms of Reference**

The objective of the scientific review is to examine the processing of sexual assault investigation kits in the Forensic DNA Analysis laboratory and provide any recommendations on process improvements that could be made.

Specifically, the review will consider the following:

- the current Standard Operating Procedure for Examination of Sexual Assault Cases
- associated Standard Operating Procedures The Acid Phosphatase Screening Test for Seminal Stains, Examination For & Of Spermatozoa and Detection of Azoospermic Semen in Casework Samples
- small report titled 'AP Paper False Positive Investigation' PAUL is this to be reviewed? It was provided in the papers that you gave me

# Documents to be provided

Current versions of the above SOPs and small report will be provided via email (Procedure for Examination of Sexual Assault Cases QIS #32106v4; The Acid Phosphatase Screening Test for Seminal Stains QIS #17186v12, Examination For & Of Spermatozoa QIS #17189v13 and Detection of Azoospermic Semen in Casework Samples QIS #17185v10)

# **Provision of work**

Assessment of the SOPs and report can be conducted remotely with any follow-up being conducted either by via email, phone, teleconference, video conference or in person.

From: Paul Csoban

**Sent:** Wednesday, 8 February 2017 7:41 AM

To: Cathie Allen
Subject: Re: Brief - version 1

Thanks Cathy

That's great. I will add further details and send off.

Great job on short notice

Paul

Sent from my iPhone

On 7 Feb. 2017, at 6:25 pm, Cathie Allen

> wrote:

Hi Paul

Here's what I achieved so far.

Cheers

Cathie

<image001.jpg> Ca

**Cathie Allen** 

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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<Brief\_for Noting\_FSS\_Forensic DNA Analysis v1.doc>

From:

Monday, 6 February 2017 9:05 AM Sent: To: John Bone Cathie Allen Cc: **Subject:** RE: Documents for Scientific Review Thanks John Happy NZ Day <sup>⊕</sup> Pal ----Original Message-----From: John Bone [mailto: Sent: Monday, 6 February 2017 9:04 AM To: Paul Csoban Cc: Cathie Allen Subject: Re: Documents for Scientific Review Thx Paul will do. Nz day here so will get Sarah et al to review and revert tomorrow. Regards John Sent from my iPhone > On 6/02/2017, at 12:00 PM, Paul Csoban < wrote: > Hi John, > As discussed, please find attached all relevant documentation for the review. > I would be grateful if you could send us the quote for this work and please feel free to call with any queries. If the queries are of a technical nature around the scientific aspects, Cathie would be the most appropriate person for (?)Sarah to call. > Regards > Paul > > [HSQ email image] > Paul Csoban > Executive Director > > Forensic and Scientific Services > Health Support Queensland, Department of Health > > > p |

Paul Csoban

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> Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and
future.
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# **Director-General Brief for Noting**

Department RecFind No:	
Division/HHS:	
File Ref No:	

Requested by: Paul Csoban, Executive Director, Forensic and Scientific Services

SUBJECT: Possible Public Interest Disclosure regarding Forensic DNA Analysis

NOTED		
MICHAEL WALSH Director-General	Date: / /	
Director-General's comment	Ministerial Brief for Approval required  Ministerial Brief for Noting required	

#### Issue/s

- Amanda Reeves, a staff member in Forensic DNA Analysis, Forensic and Scientific Services wishes to return to her substantive role as a Senior Court Reporting Scientist, after a period of extended leave.
  - 1.1. Ms Reeves raised an issue to her line manager, Mr Justin Howes regarding a process used to examine sexual assault investigation kits on the 4<sup>th</sup> of March 2016.
  - 1.2. A project was instigated into this issue and feedback on the project to the Forensic DNA Analysis Management Team began on the 12<sup>th</sup> of May 2016.
  - 1.3. On the 9<sup>th</sup> of June 2016, during a Forensic DNA Analysis management team meeting, Mr Allan McNevin behaved inappropriately towards Ms Reeves when discussion commenced regarding the project (called Project #181).
  - 1.4. Mr McNevin has apologied twice to Ms Reeves for his outburst. Ms Reeves and Mr McNevin were offered formal meditation and a facilitated discussion. Mr McNevin agreed to both options and Ms Reeves declined both options.
  - 1.5. The matter has been appropriately dealt with at a local level. In addition to the management at a local level, an external investigation into the incident at the management team meeting has commenced. Mark Brady, Principal Consultant of Livingstones has conducted interviews and is preparing a final report.
  - 1.6. Ms Reeves has been on extended leave from the 30<sup>th</sup> of November 2016, with a return to work date of the 31<sup>st</sup> of January 2017.
  - 1.7. Ms Reeves engaged JA Hodgens, Principal, Human A.S.S.E.T. Solutions in early December 2016 to act on her behalf.
  - 1.8. Ms Reeves, through her lawyer, has threatened a Public Interest Disclosure (PID) on the issue regarding processing of sexual assault investigation kits.
  - 1.9. Risk mitigation steps have been introduced into the process used to examine sexual assault investigation kits. Additionally, a scientific review into the processing of sexual assault investigation kits has commenced, with the Institute of Environmental Science and Research (ESR), New Zealand's Crown Research Institute.

Department RecFind No:	
Division/HHS:	
File Ref No:	

- 1.10. On the 6<sup>th</sup> of February, 2017, Acting Superintendent Scott McLaren, Forensic Services Group, was been briefed regarding the possible PID. A/Supt McLaren advised that he would prepare a briefing for the Assistant Commissioner Michael Condon.
- 2. Ms Reeves has obtained medical clearance to return to her duties and wishes to be placed back in her sustantive role, which requires reporting and expert evidence on sexual assault cases, among other case types. An offer of alternative employment has been extended to Ms Reeves until both the external investigation and the scientific review have been completed, however Ms Reeves is insistent on returning to her substantive role.
- 3. If Ms Reeves is returned to her substantive role prior to the conclusion of the external review and scientific review, she may be called to provide expert testimony on a sexual assault case. Ms Reeves would be under oath and be required to truthful answer questions relating to the processing of sexual assault investigation kits when she has highlighted her misgivings in the processing. This would be detrimental to Ms Reeves and the work unit.
- 4. If Ms Reeves were to provide evidence that processing of sexual assault evidence was inadequate, this would be detrimental to Queensland Health, the Queensland Police Service, the Queensland Government and the community would lose faith in the scientific work that is conducted in the forensic areas of Forensic and Scientific Services.
- 5. It is recommended that Ms Reeves undertaken alternate duties until the outcomes are known for the two reviews currently underway.
- 6. Human Resources and Legal advice is being sought on this issue.
- 7. Ms Reeves was deemed fit for return to work from the 2<sup>nd</sup> of February 2017.

#### **Background**

- 8. Forensic DNA Analysis are delegated the authority to test forensic items by the Commissioner of Police through the Police Powers and Responsibilities Act 2000 (section 488B). The work unit analyses approximately 21,000 crime scene items and 15,000 person samples each year. Results are provided electronically to the Queensland Police Service (QPS). Statement of Witness documents and expert evidence are provided to all levels of the Queensland Courts. Forensic DNA Analysis provide vital DNA analysis results for both court purposes and intelligence purposes, which enable the QPS to link together previously unrelated alleged offences.
- 9. Forensic and Scientific Services underwent a Ministerial Taskforce Review in 2005 following three front page media articles highlighting the large number of untested items held by Forensic DNA Analysis. The QPS and FSS worked collaboratively to reduce the number of untested items down to zero and begin working in real-time at the beginning of 2008. The Review also provided resources for the purchase of automated platforms to assist with laboratory throughput, a laboratory refurbishment and additional staff members (both permanent and temporary) to process the items.

#### **Results of Consultation**

10. Mr Jade Franklin, Manager Huamna Resources and Business Relationships has been consulted during this process. Who from Legal has been consulted?

#### Resource Implications (including financial)

- 11. The report from Livingstones is estimated to cost approximately \$20,000.
- 12. The scientific review from ESR is estimated to cost approximately \$2,500.

#### **Attachments**

Department RecFind No:	
Division/HHS:	
File Ref No:	

# 13. Please ensure all attachments referred to are included and numbered.

Note – three levels (only) of internal approval are required; the table below will be removed prior to submission of brief to Minister

Author	Cleared by: (SD/Dir)	Content verified by: (CEO/DDG/Div Head)
Paul Csoban	Sharon Kelly	<name></name>
Executive Director	General Manager	<position></position>
Forensic and Scientific Services	Community and Scientific Support	<unit hsd=""></unit>
		<tel number=""></tel>
		<mob number=""></mob>
8 February 2017	<date></date>	<date></date>

From: Paul Csoban

**Sent:** Wednesday, 8 February 2017 7:41 AM

To: Cathie Allen
Subject: Re: Brief - version 1

Thanks Cathy

That's great. I will add further details and send off.

Great job on short notice

Paul

Sent from my iPhone

On 7 Feb. 2017, at 6:25 pm, Cathie Allen <

wrote:

Hi Paul

Here's what I achieved so far.

Cheers

Cathie

<image001.jpg> Cathie Allen

Managing Scientist - Police Services Stream

Forensic & Scientific Services,



HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

<Brief\_for Noting\_FSS\_Forensic DNA Analysis v1.doc>

Enquiries To:

Jade Franklin

Manager HR and Business Relationships People Performance and Excellence

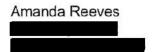
Telephone: File Ref:

HSSA/003129



Health Support Queensland

Department of Health



#### Return to work

I refer to your letter to Mr Paul Csoban, Executive Director, Forensic and Scientific Services dated 5 February 2017 and your previous correspondence and discussions regarding your recent return to work request.

I have discussed this matter with Mr Csoban and Mr Jade Franklin, Manager Human Resources and Business Relationships and reviewed the relevant correspondence in relation to this matter.

I will now consider each of the matters you raised in your letter dated 5 February 2017.

# Grievance against Alan McNevin

In relation to the investigation into the matters you have raised against Mr Allan McNevin I note the following:

- (a) HSQ is satisfied that Ms Whelan and Ms Brisotto undertook the appropriate local management action to investigate the matters you raised in relation to the incident which occurred on 9 July 2016. As outlined in the letter to you from Mr Csoban dated 3 February 2017, Mr McNevin's conduct was addressed by Ms Whelan promptly. This, in combination with the offer of a mediation process and a facilitated discussion between yourself and Mr McNevin, was determined an appropriate response to the matters you had raised.
- (b) In response to your question raised in your letter dated 5 February 2017 regarding "why Mr McNevin remained within the workplace" and you wanting to understand whether it was "normal practice to suspend or move a subject officer from the workplace". I note you were not suspended or moved out of the workplace. Your absence from the workplace was as a result of your health.

Health Support Queensland

Website www.health.qld.gov.au

Since you have received medical clearance, our concern has been to return you to your position provided it is safe, and is reasonably practical to do so.

- In response to the matters you have raised regarding suspending or removing Mr McNevin from the workplace. We did not move Mr McNevin because in our view it was not appropriate or necessary to protect you, Mr McNevin or other employee's health and safety. Again, as stated in the letter from Mr Csoban dated 3 February 2017 "I remain satisfied with all steps taken to date by Ms Whelan and Ms Brisotto to attempt to resolve the matter at a local level". Further, other than to the extent that it impacts on your immediate working environment, what steps are taken in relation to Mr McNevin's employment are a matter for HSQ and Mr McNevin and are personal to him. They are not a matter for discussion with you.
- (d) The Livingstones investigation is an independent investigation into the concerns you have about Mr McNevin. This independent investigation was initiated by Mr Paul Csoban. As previously advised, the final report from Livingstones is yet to be received by HSQ and as such I confirm that HSQ has not "formed a view", but, as advised in the letter to you from Mr Csoban dated 3 February 2017 "at this stage the outcome of the independent investigation is still pending and in the absence of a finalised process I do not have a valid reason to remove Mr McNevin from his substantive position". As outlined above, I am satisfied the measures taken in the interim ensure that there is no risk to the health and welfare of any employee, including yourself and Mr McNevin.
- (e) In relation to the findings of the Livingstones report, HSQ will consider the findings of the report and recommendations and take appropriate management action as appropriate. I wanted to take this opportunity to clarify that the findings of the report will not be "tabled" with you or be provided with "clarification as to why their actions were deemed appropriate or not" as suggested in your letter. HSQ will review the findings of the Livingstones report and take action it considers appropriate, including meeting with you to discuss any aspect that impacts you as HSQ considers appropriate.

#### Issues raised with the Scientific Process

I have been advised that concerns with the integrity of the scientific tests that are undertaken in relation to testing semen samples which could affect the outcome of criminal proceedings relating to sexual assault cases, were first raised by members of your team in or around March 2016. I am also aware that you then escalated these concerns. I note that you provided additional documents to Mr Csoban in a meeting on 19 January 2017 in relation to your continued concerns regarding the integrity of scientific tests. Thank you for raising these concerns, it was proper and appropriate for you to do so and for providing us with further documentation.

After the initial complaint was made in March 2016, Forensic and Scientific Services (**FSS**), considered alternative processes to conducting the test to ensure the veracity of the testing

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Health Support Queensland

which included a series of quality assurance tests to confirm the reliability of the testing regime. This process, as you are aware because you were directly involved was named Project #181. A slightly modified testing procedure was introduced in or around August 2016.

The modified testing procedure was implemented after consultation and is based on the risk assessment and quality assurance processes of the FSS procedures. No concerns with the testing process have been raised by the 'collective group' including your direct colleagues since that process was undertaken.

However, since the introduction of the modified testing method, you have raised a number of concerns with the process on the following occasions:

- (a) during a telephone conversation between Mr John- Anthony Hodgens (your legal representation) on 8 December with Mr Franklin. Mr Hodges referred to the fact that it was open for you to make a public interest disclosure application regarding the testing regime;
- (b) in a meeting on 19 January 2017 with Mr Franklin and Mr Csoban, which you advised you still had "concerns" regarding the testing regime;
- (c) during a telephone conversation with Ms Frederiksen, Principal Adviser, Safety and Wellbeing on 24 January 2016. This conversation was regarding your return to work, during which you provided Ms Frederiksen with an understanding of what a suitable duties plan could include. Your proposed plan included conditions which included not attending court and not undertaking work related to sexual assault cases that needed semen screening.

Whilst I acknowledge that you have now, in your letter dated 5 February 2017, advised HSQ that you are confident in undertaking the full scope of your role (on the basis that other employees are confident in the modified testing process). This is not consistent with your previous position as outlined above.

#### Your email to Paul Csoban on 7 February 2017

I confirm that you first notified HSQ of your desire to return to work on 19 January 2017 in a meeting with Mr Franklin and Mr Csoban. In this meeting Mr Franklin confirmed the requirement that you obtain medical clearance. You initially obtained medical clearance for 3 days work and subsequently for 5 days work.

Since you first notified HSQ of your desire to return to work HSQ has been working with you, and your treating medical practitioner via Ms Frederiksen to facilitate your return to work.

You provided notice of your return to work on 19 January 2017 after being on leave for approximately 2 months. The suggested conditions of your return to work you discussed with Ms Frederiksen on 24 January 2017. These conditions were outlined in Mr Csoban's correspondence dated 3 February 2017. HSQ carefully considered the conditions you

requested however it was determined that it was not operationally viable and administratively would have been difficult to implement.

# Direction pending outcome of Scientific Report

Your role has significant obligations to the Court including that you provide honest and independent expert evidence. We are extremely concerned that despite your most recent comments you are fundamentally unable to give expert evidence until you have taken additional steps to verify your concerns about the scientific process and satisfy yourself accordingly. We consider the external expert that will undertake a further scientific investigation and provide a report (scientific report) will assist you in alleviating the concerns you have regarding the integrity of the testing. Until such time as those additional steps are taken, in my view you cannot in good conscience give professional expert evidence to the Court and HSQ will not place you in such a position of potential conflict.

Given the concerns outlined above, I direct you not to undertake any duties which involve the reporting on semen testing or any related duties until such time as the outcome of the scientific review is provided and HSQ has had an opportunity to consider the outcomes identified in the report. In the interim, I direct you to participate in a temporary role participating in scientific research which, on review of your role, responsibilities and position description is within your expertise, role and classification. You would undertake this task full time at your same classification and pay rate and will be located at the Coopers Plains Campus, 39 Kessels Rd. If you would like further information about this position, please contact Mr Csoban directly.

As a gesture of good faith I have on this occasion decided to pay you from the date of your first medical clearance which was on 18 January 2017.

#### **Next steps**

Please advise Mr Franklin as soon as possible, that you have read and understood the contents of this letter and will comply with the direction. Mr Franklin can be contacted on

I appreciate that this may be a difficult time for you. Employee assistance offers a confidential counselling service which is free of charge to all employees of HSQ for up to six sessions per calendar year. Access to this service is by self-referral. Please contact Optum on . More information on employee assistance can be found at <a href="http://qheps.health.qld.gov.au/eap">http://qheps.health.qld.gov.au/eap</a>.

Mr Gary Uhlmann
Chief Executive Officer

10 | 2 | 2017

**Health Support Queensland** 

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# External Investigation into DNA Analysis Team – Forensic and Scientific Services

Health Support Queensland

Gary Uhlmann

Chief Executive

Mark Brady

Principal Consultant

- | Employment & Industrial Relations
- Human Resources & Relationships
- Organisational Advisors & Psychologists



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# **Investigator Précis**

# **The Organisation**

Livingstones is one of the largest Workplace Relations and Human Resource Management Consultancy firms within Australia. We operate on a national basis from our Brisbane office offering the services of our 23 professional Consultants. Our Industrial Relations division acts on behalf of employers (Private Sector, Local Government and State Government Departments) on all aspects of employee relations including, but not limited to, investigations, mediation, advocacy and training.

The investigator was Mark Brady, Principal Consultant of Livingstones.

# **Mark Brady**

Mark's extensive experience across a broad range of employee relations means that he can provide expert and practical advice and services on all aspects of workplace matters. His experience includes advocacy, investigations and resolving complex employment matters.

Mark is a specialist in complaints management, managing and resolving misconduct and workplace bullying complaints as well as providing strategic advice in relation employment arrangements and conditions. Throughout his career, Mark has been recognised for providing creative and practical solutions for complex workplace issues. Mark is also an experienced mediator and brings to his work a genuine desire to reach resolution in a constructive manner where possible, however he does not shy away from the 'hard' issues when the need arises.

Mark works closely with clients to ensure that the strategic solutions support the business needs and philosophy of the client whilst addressing the specific issues that arise in the workplace and any systemic contributors.

# Context

On 24 October 2016, Livingstones was appointed by the Chief Executive Officer, Heath Support Queensland in accordance with the *Hospital and Health Boards Act 2011* to investigate and report on matters related to the management team of Forensic DNA Analysis at Forensic and Scientific Services as outlined in the Terms of Reference. This arises from an incident on 9 June 2016 between Allan McNevin and Amanda Reeves at the management team meeting. Both Mr McNevin and Ms Reeves are supervising scientists of their respective teams and members of the Forensic DNA Analysis management team.







# **Methodology**

A standard investigative process was utilised in which the principles of 'natural justice' were observed.

Persons considered to be in a position to provide relevant evidence for the investigation were requested to attend an interview and advised of their option to have a support person present. Each interviewee was provided a written statement arising from the interview and given an opportunity to request amendments to ensure this was accurate and complete. Signed statements were taken from the following witnesses and are appended to this report:

Interviewee	Attachment No	Date of Interview
Amanda Reeves	"A"	16/11/16
Allan McNevin	"B"	17/11/16
Deborah Whelan	"C"	22/11/16
Justin Howes	"D"	28/11/16
Paula Brisotto	"E"	29/11/16
Kristen Scott	"F"	12/01/17
Claire Gallagher	"G"	17/01/17
Jacqui Wilson	"H"	17/01/17
Janine Seymour-Murray	"ļ"	18/01/17
Kerry-Anne Lancaster	"J"	17/01/17
Kevin Avdic	"K"	18/01/17
Kylie Rika	"L"	17/01/17
Michelle Margetts	"M"	18/01/17
Nicole Gardiner	"N"	18/01/17







Pierre Acedo	"O"	17/01/17
Rhys Parry	" <b>P</b> "	17/01/17
Thomas Nurthen	"Q"	18/01/17
Valerie Caldwell	"R"	17/01/17
Wendy Harmer	"S"	17/01/17
Abigail Ryan	"Т"	18/01/17
Adrian Pippia	"Џ"	17/01/17
Angelina Keller	"γ"	18/01/17
Cassandra James	" <b>W</b> "	18/01/17
Chelsea Savage	"X"	18/01/17
Cindy Chang	«γ»	18/01/17
Allison Lloyd	"Z"	23/01/17
Sharon Johnstone	"AA"	23/01/17
Angela Adamson	"AB"	17/01/17
Email from Justin Howes	"AC"	19/01/17

All parties involved have been verbally reminded not to verbally or physically, overtly or covertly victimise in any manner, including career issues, any person involved in this matter.

All parties involved have been verbally reminded to maintain a high level of confidentiality in relation to the process and issues surrounding this matter. All parties involved have also been verbally reminded that any breaches of confidentiality may result in disciplinary action being taken against them

# **Terms of Reference**

The Terms of Reference, as issued by HSQ CEO Gary Uhlmann dated 24 October 2016 specified that the investigator is to investigate matters relating to the allegations regarding inappropriate workplace behaviour. More specifically:





- (a) review and investigate Ms Reeves allegation that Mr McNevin's response to feedback provided by her in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours;
- (b) review and investigate Ms Reeves allegation that Mr McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships;
- (c) review and investigate Ms Reeves allegation that an email from Justin Howes, Team Leader to all reporting staff on the 5 of August 2016 regarding standardised statement wording was specifically directed at Mr McNevin;
- (d) review and investigate Ms Reeves allegation that Mr McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide;
- (e) review and investigate Ms Reeves allegation that Mr McNevin caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so:
- (f) review and investigate Ms Reeves response to Mr McNevin's email apology on the 9th of June 2016;
- (g) review and investigate the poor working relationship between the substantive team members of the Forensic DNA Analysis management team and provide details on the areas of improvement;

The Health Service Investigator is to make findings and recommendations in respect of:

- (a) the matters outlined in 3.1 above or any other relevant aspect of the complaints;
- (b) possible misconduct by any employee of Health Support Queensland;
- (c) any identified systemic weaknesses in Forensic and Scientific Services, Queensland Health, or Health Support Queensland policies, procedures or processes and provide any recommended remedial or preventative actions, In particular the management of interpersonal relationships between the management group within Forensic DNA Analysis;
- (d) the ways in which the management, administration or delivery of the public sector health services, including employment matters, can be maintained and improved; and
- (e) any other matter identified during the course of the Investigation.

# **Standard of Proof**

As with any administrative investigation, the standard of proof applied to the assessment of the evidence is the "balance of probabilities".

The following principles as set down in the seminal case of Briginshaw v Briginshaw, High Court of Australia (1938) 60 CLR 336, have been taken into consideration when making findings in this investigation:

"The seriousness of an allegation made, the inherent unlikelihood of an occurrence of a given description, or the gravity of the consequences flowing from a particular finding are considerations which must affect the answer to



the question whether the issue has been proved to the reasonable satisfaction of the tribunal. In such matters, 'reasonable satisfaction' should not be produced by inexact proofs, indefinite testimony or indirect references."

"When in a Civil proceeding, a question arises whether a crime has been committed, the standard of persuasion is, according to the better opinion, the same as upon other Civil issues....but, consistently with this opinion, weight is to be given to the presumption of innocence and exactness of proof is expected...."

# **Policy Framework**

If substantiated, the allegations as made, may constitute a breach and/or failure to comply with the following policies, procedures and/or legislation:

Code of Conduct for the Queensland Public Service

# **Identification of Allegations**

The specific allegations that are subject to this investigation are as follows:

- 1. Amanda Reeves alleges that Allan McNevin's response to feedback provided by her in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours.
- 2. Amanda Reeves alleges that Allan McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships.
- 3. Amanda Reeves alleges that an email from Justin Howes, Team Leader to all reporting staff on the 5 of August 2016 regarding standardised statement wording was specifically directed at Mr McNevin.
- 4. Amanda Reeves alleges Allan McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide and caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so.







# Consideration of Evidence

# Allegation 1

Amanda Reeves alleges that Allan McNevin's response to feedback provided by her in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours.

#### **FINDING: Not Substantiated**

#### Overview

The incident is alleged to have occurred at the Foresnic and DNA Analysis Management Team meeting on 9 June 2016. All attendees at the meeting were interviewed by the investigator in relation to this meeting and the alleged incident.

The interviewees were:

- Amanda Reeves
- Allan McNevin
- Deborah Whelan
- Justin Howes (Chair)
- Kirsten Scott
- Kylie Rika
- Kerry-Anne Lancaster
- Sharon Johnstone
- Wendy Harmer
- Pierre Acedo
- Allison Lloyd

# **Available Evidence**

#### **Amanda Reeves states:**

At interview, Ms Reeves outlined the following, "On 9 June, the topic came up as it was on the Management meeting agenda. I again was addressing the meeting about what I perceived to be a risk for the organisation. I was seated immediately next to Allan at a horseshoe table. I was sitting about 40cms away from Allan. Allan is about 6 feet tall and trained in martial arts."

Further, "I can't remember Allan's exact words but I saw his arms raised and then brought down onto the table with a noticeable and audible impact. In the same movement he pushed himself back from the table and turned toward me whilst yelling "Oh for God sake Amanda, I'm aware of the risks, you keep telling me....." I believe he continued yelling for some time but I didn't recall what he said after that as I was in shock."

Ms Reeves further outlined, "I noticed Deb Whelan jump, and Justin Howes and Deb both said repeatedly, "Allan stop, Allan stop, Allan stop". I just sat there as it took me moment to work out that I wasn't in physical danger. Nobody asked Allan to leave. Deb Whelan appeared flustered and said we should move on and then started talking about the next agenda item. I then asked to leave the room and I left. After a period of time I returned and sat until the meeting was finished."



Further, "I left the meeting but Allan was asked to remain behind. The meeting minutes do not reflect this incident at all. There is a reference to 'stress in the lab' under 'New Business'. I then received an email from Allan at 10.33am (AR12) in which, rather flippantly in my opinion, Allan apologised for 'spitting the dummy at me'. I responded at 11.00am acknowledging his apology and letting him know, as the recipient of his behaviour, how it made me feel. I wouldn't meet with Allan because he had just physically and emotionally intimidated me and I didn't feel safe. When I left the meeting, I went and sat in a room and fell apart."

Ms Reeves also added, "The behaviour by Allan McNevin on 9 June 2016 is an escalation of previous behaviour by Allan towards me, in my opinion. I believe that Allan dismisses what I have to say. I think Allan has trouble dealing with me as an assertive woman. While we have equal standing at the management table, I feel that unless my opinion gels with Allan's he dismisses me out of hand."

Further, "I have raised concerns with my line manager Justin Howes (HP6 Forensic Reporting & Intelligence Team - FRIT) about this in the past. As a result of this, I met with Allan to address the issues. This meeting was a couple of years ago. Allan seemed to listen to me at this meeting but in my opinion his behaviour did not change following this. There have been other attempts to address this through my line manager."

#### Allan McNevin states:

At interview Allan McNevin outlined, "At the management meeting on 9 June, I was going to present my proposal to investigate the issue. The matter wasn't being discussed so I thought I would mention it so people were aware of what was happening. Once again, Amanda raised the issue that it was a risk and did so a number times in the discussion. She wasn't adding anything to the discussion, just saying it was a risk. I wanted to look at the issue and find out where we are going wrong rather than jumping straight to a solution. I think we needed to find out what was going wrong in the process rather than jumping to a solution. Amanda then again raised that sperm was going missing."

Further, "That is when I lost my temper and shouted something like, "I'm not stupid, I understand that there is risk." I didn't swear. Amanda was sitting beside me and I turned and said it to her. I don't recall exactly my physical actions but I might have pushed myself away from the table. I do recall turning to face her as she was sitting beside me."

Mr McNevin further outlined, "I certainly didn't intend to do anything that was physically intimidating. Straight away, I knew I had done the wrong thing. It all happened very quickly. Either Deb Whelan or Justin Howes was chairing and I remember they both interjected and said something to calm the situation. Shortly after that, Amanda took her phone and left the room."

Further, "The meeting continued and then when the meeting was nearly finished Amanda returned. While Amanda was gone, I was thinking about apologising and how I could say it. I knew she would be upset and I know how she had been upset in the past. The meeting concluded. Before I could say anything to Amanda she got up and left."

Mr McNevin further outlined, "The different teams approach issues from different positions so sometimes there is conflict in the management team. This is normal for this sort of work. "I like to approach problems from a very scientific position whereas Amanda can get very emotional. She says things like 'you have to remember that there is a person on the end of this.' It seems that Amanda and I often end at opposite ends of the argument when issues are raised. I never feel it is personal it is just the way the discussion pans out as we come from different angles sometimes with competing interests."

Further, "Amanda has a strong personality. She can often go straight on the front foot about a matter. She can be quite persistent when she wants to put a point forward. It can be very frustrating in that Amanda can labour the same point over and over again without bringing new information to the discussion. I think over the years, I



have managed my discussions with Amanda very well. I have been commended on this at times by others on the management team. I have worked on different strategies to deal with this. Sometimes, I will disengage when I can see the conversation is going nowhere. Sometimes I will sit back from the table and doodle in my book rather than engage in the conversation."

#### **Deborah Whelan states:**

Deborah Whelan is the Managing Scientist for the Coronial Services stream at Forensic and Scientific Services. Ms Whelan was relieving as the Managing Scientist for Police Services which includes the DNA Analysis Team while Cathie Allen was on leave. At Interview Ms Whelan outlined, "I wasn't aware of any issues between Amanda Reeves and Allan McNevin until the management meeting of the Forensic DNA Analysis on 9 June 2016. I was present at this meeting. I recall at this meeting, there were a number of people there who were backfilling positions and others who were regular attendees. The meeting was going along in a regular way until we got to the point where staff talk about projects they were working on."

Further, "I recall Allan gave an outline of his approach to his project which I think was #181. During this time, Amanda Reeves began to voice her concerns to Allan about the design of the project. Amanda was frustrated and she was making her point over and over again to Allan as if she wasn't being heard by Allan. Allan then shouted at Amanda. I can't specifically recall exactly what Allan shouted. I don't recall whether Allan waved his arms or hit the desk when he shouted."

Ms Whelan further stated, "At this time, Justin Howes, who was the chair of the meeting and I attempted to shut this down, I think I said 'stop' to Allan when he raised his voice. I planned then I was going to meet with Allan after the meeting to talk to him about this his behaviour rather than in front of everyone at the meeting. I tried to move the agenda on from this point. At the time, the shouting made the biggest impression on me. I didn't notice anything that was physically intimidating."

Further, "As I was focused on getting Allan to stop, I didn't notice anything particular about Amanda until she left the meeting. I saw Amanda leave the meeting, which I assumed was to remove herself from the situation. In hindsight, I think she was upset. I don't recall Amanda coming back into the meeting. I think I may have said to Justin that we need to speak to Allan after meeting. I don't recall anyone else at the meeting saying anything about the incident."

# Justin Howes states:

Justin Howes is the Team leader for Forensic Reporting and Intelligence Group. Mr Howes is Ms Reeves' line manager. At interview Mr Howes outlined, "I chaired the meeting on 9 June. Amanda and Allan were sitting next each other. Amanda was asking a number of questions of Allan which he didn't have the time to reply. Amanda was unable to answer what she was after out of the review. Her response was that she "just wanted it fixed." There were a number of questions from Amanda to Allan."

Further, "Allan then placed two hands on the table and pushed himself back. He then yelled at Amanda something like "Amanda do you think I'm stupid." I then said to Allan to stop. He said something else but I missed it. Deb Whelan then said to move the agenda on."

Mr Howes further outlined, "Allan was loud when he said it but I wouldn't describe it as physically intimidating. There certainly wasn't any physical threat. At then end of the meeting, I asked Allan to stay behind. Deb Whelan, Kirsten Scott and I met with Allan afterwards."

Further, "I met with Allan and told him that I couldn't control how Amanda feels. I outlined that Amanda said the feelings were a result of many events and that she didn't feel heard or respected. I told him to be careful with his laid back mannerisms



as it can look like he was dismissive. I told him he should be more active in his listening and he accepted that. I outlined that Amanda wanted to work with Allan and have robust discussions but she didn't feel she was being listened to. Allan said he would be happy with an email from Amanda saying that it was just her perception and that she acknowledged that he wasn't that sort of person. He committed to work together."

#### **Kirsten Scott states:**

Kirsten Scott is a Senior Scientist, Quality and Projects. At the time, Ms Scott was acting Team Leader, Evidence Recovery and Quality. Mr McNevin reports to this position. At interview Ms Scott outlined, "I recall the management team meeting on 9 June 2016. It started off as a normal management team meeting. I knew that Allan McNevin was going to raise project #181 as I had been working with him to look at a reasonable approach to deal with the issue. At the time, I was acting as the team leader."

Further, "As soon as Allan raised project #181, I knew it wasn't going to go well. As soon as he started to speak, I noticed Amanda Reeves' body language. It was very aggressive, she was frowning while Allan spoke. It was a look of displeasure at everything he said. From memory, Amanda was sitting next to Allan and I was sitting across from them both. As soon as Allan finished, from my point of view, Amanda's response was very confrontational and disproportionate. Allan had been presenting the information in a very calm way and I could see Amanda's tension building while Allan was talking. When Amanda did speak, it was a very emotional and intense response."

Ms Scott further outlined, "I don't remember the details of what Amanda said other than she disagreed strongly with the proposed approach. I don't recall exactly what Amanda's approach was but I recall that she wanted to deal with the whole issue straight away whereas Allan wanted to establish a baseline so that it could be used to compare results. Scientifically, it shouldn't have been an issue. Both approaches were scientifically valid approaches but during the discussion, Amanda had a very emotional response to what was essentially a scientific discussion." The conversation went to and fro and became more intense. Amanda was saying that Allan didn't understand the consequences of the issue but Allan did understand and was telling Amanda that."

Further, "The conversation was making no progress scientifically. Allan then raised his voice at Amanda and pushed himself back from the table. I don't recall what Allan said. Allan may have hit the table with his hands and he pushed himself back but he didn't thump the table with his hands or anything like that. Allan's voice was raised above the way he normally spoke. I wouldn't describe it as yelling as Allan is a big man with a loud voice and could have been a lot louder. I would describe it as about 50% louder than he normally spoke."

Ms Scott further stated, "From my point of view I didn't see anything physically intimidating by Allan in the incident. I remember when I saw the allegation later that it was physically intimidating, I was shocked. Deb Whelan then spoke and told Allan to be quiet. Then, within moments, Amanda left the meeting. I don't recall if Amanda returned to the meeting. At then end of the meeting, Deb Whelan asked Allan, Justin Howes and me to remain behind."

Further, "I have never noticed or had any concerns about Allan's behaviour in the past. He is very calm and collaborative in his style. I have not observed that he behaved any differently towards Amanda that he had with anyone else. In my view, Allan's personality trait of being laid back may have contributed as Amanda may not have thought he was concerned about the matters raised when I believe he was concerned but not at same heightened emotional state as Amanda."







# Kylie Rika states:

Kylie Rika is Senior Reporting Scientist in the DNA Analysis Team. At interview Ms Rika outlined, "I recall the management team meeting on 9 June 2016. It was quite calm until we started discussing the project #181. Allan McNevin, who was managing the project was giving his report. Amanda Reeves was asking him questions. I don't think Amanda believed Allan understood what she was trying to say so she said it in a couple of different ways."

Further, "Allan then slammed his hands on the table and pushed his chair back. He then yelled something like, "I'm not stupid Amanda, I know what you are saying." I was sitting next to Amanda who was sitting next to Allan. I jumped as it shocked me. I also noticed some others being shocked by this. At this time, either Justin Howes or Deb Whelan, it may have been both then intervened and said something like, 'that's enough Allan.' The meeting continued and we parked the topic."

Ms Rika further outlined, "I would describe Amanda's questioning as being passionate as from my perspective; Amanda was passionate to ensure the project addressed all the issues. As it a topic she was passionate about. She wanted to ensure the team got the best of out of the project. I don't think Amanda was trying to provoke Allan but was showing concern about the project but being robust about her concerns."

Further, "I remember that after the meeting I was still shaking. I felt intimidated by the incident. I remember saying this to my boss Justin Howes and said I hope it never happens again. After Allan was told to stop, Justin was running the meeting and tried to move on. Amanda stayed for about a minute and then left the meeting. Amanda was away from the meeting for about half an hour and then she came back. Nobody went after Amanda after she left."

Ms Rika further outlined, "There has been tension between Amanda and Allan for a while. I think that both of their communication styles are not conducive with each other and this causes friction. Allan's communication style is very relaxed. At the management meetings he sits back and appears very nonchalant. Sometimes I personally feel, my perception of the way he communicates to Amanda is that he can be dismissive. In my view, when Amanda is talking, he comes across as dismissive or what Amanda is saying is a hassle. Sometimes I feel Allan acts like this to me but not as much. I haven't witnessed any behaviours from Allan that I would describe as aggressive or inappropriate."

# **Kerry-Anne Lancaster states:**

Kerry-Anne Lancaster is a scientist in the Quality and Projects Team. At interview, Ms Lancaster outlined, "I recall at the management meeting on 9 June 2016, there was tension between Amanda Reeves and Allan McNevin in relation to a project which I had no involvement with. Part of the work of the quality team is to take care of the paper work after the project finished, we don't get involved during the project."

Further, "At the meeting I recall there was a heated discussion between Amanda and Allan. I don't remember the specific details but I recall the word stupid being used. I'm not sure if someone said that someone was stupid. I don't really remember. I remember there were raised voices. I wouldn't call it yelling, more frustrated. I think Allan was talking in a loud voice. I remember Allan was standing, he might have been writing on the whiteboard or something like that. There may or may not have been hitting hands on the table, I don't really remember. I remember that Amanda was sitting near Allan, the whiteboard was near her. I don't remember if I saw anything I would describe as physical intimidation."

Ms Lancaster further outlined, "Almost immediately, Amanda left the room very upset. I saw her later in the corridor crying after the meeting as I left. I may have said something to Amanda like, 'Are you okay' but I don't specifically remember. Kylie Rika may have been comforting her at the time. To me, it was an argument that was heated. I have never been in a management team meeting where the discussion had become so heated. I



believe Amanda and Allan may have had some disagreements in the past but nothing that has come to my attention. I have been here for about 12 years."

#### **Sharon Johnstone states:**

Sharon Johnstone is a Senior Scientist in the Intelligence Team. At interview Ms Johnstone outlined, "I am a member of the management team. I attended the management team meeting on 9 June 2016. I remember Allan McNevin losing his temper. I don't remember the exact discussion. There was quite a detailed discussion between Allan and Amanda Reeves."

Further, "I believe there was some miscommunication between the two of them. Amanda was repeating herself over and over again. I would describe it as insistent. I think Allan understood what Amanda was saying but Allan didn't believe Amanda was taking into account what Allan was saying."

Ms Johnstone further outlined, "I remember thinking that I would have pulled up the conversation before it got to the point it did but it wasn't pulled up. I remember Allan then raised his voice and banged on the table with his fists. He said something like, "I know Amanda. I'm not stupid" It was quite loud and it surprised the room. I would call it yelling. It was a clear display of frustration. Justin Howes, who I think was chairing and tried to calm the situation down. We did move on to the next topic of conversation. Amanda was shocked at first, think everybody was shocked. After a minute or two Amanda left the meeting. I don't remember anything of note after that."

Further, "I don't believe Allan was being physically intimidating towards anyone in particular. He wasn't facing anyone. The room itself is in a 'U' shape. He was on the same side of the table as Amanda. I don't remember if they were sitting next to each other or whether there was one person in between them. He was more facing the way he was sitting rather than displaying his emotion directly towards Amanda."

Ms Johnstone further outlined, "It was very out of character for Allan to act like that. Allan is usually very level, he does come up with 'out of the box' ideas so he does tend to talk a lot but he rarely shows any emotion. Allan is really friendly, his whole team loves him. He has managed a number of staff over the years and does a really good job of it."

# Wendy Harmer states:

Wendy Harmer is the Administration Support Officer for the Managing Scientist, Ms Cathie Allen. At interview Ms Harmer outlined, "I regularly attend the management team meetings, in years past, I did the minutes. Now, one of the team leaders chairs the meeting and the other takes the minutes. I recall on 9 June 2016 that I attended the management meeting and Justin Howes was the chair. I have the minutes for that meeting. I recall that at the meeting, we just went through the agenda as per normal."

Further, "I remember project #181 was discussed. I recall that I was sitting nearest to the door and Allan McNevin was sitting next me. Either Amanda Reeves or Kylie Rika was sitting next to Allan but I am not sure who was. The discussion was quite intense. Allan's and Amanda were discussing their thoughts. I felt the conversation was escalating. In my opinion, Amanda was very persistent in her responses towards Allan. I'm pretty sure Allan said, "I'm not stupid Amanda" in response to what she was saying. As it was escalating, I was surprised the chairperson did not intervene."

Ms Harmer further outlined, "Allan banged his hands down on the table. It startled me. In my view it was a reaction to Amanda being persistent. Allan may have got up and left the meeting for a minute after this. Allan used a normal voice, if anything, it was just frustration. He didn't







scream or anything like that. It's a long time ago but that's my recollection. I wouldn't describe Allan's actions as physically intimidating. As I was sitting next to Allan, it startled me."

Further, "I am not aware of any issues between Allan and Amanda prior to this incident. I have had a number of dealings with Allan. He comes to see me about HR matters. I have never seen anything from him I would describe as aggressive or inappropriate. He is a lovely man and always speaks to me very nicely. I have never heard a bad word about Allan."

#### Pierre Acedo states:

Pierre Acedo is an Analytical Scientist in the Analytical Team. Mr Acedo outlined at interview, "I was present at the management team meeting on 9 June 2016. I don't usually attend but was relieving in Luke Ryan's position. This was my first management team meeting. I remember there was a discussion between Allan McNevin and Amanda Reeves about a particular experiment that Allan wanted to do. The conversation went back and forth and became heated. Allan then just blew up. This was a surprise as I had worked under Allan before and hadn't seen him like this before."

Further, "It was a while ago but I remember Allan threw his hands up in the air and said something like, 'Yes I know that Amanda, you don't have to keep throwing it my face.' I don't remember the exact words. Allan was frustrated, it was just a normal debate, but Allan acted uncharacteristically and yelled at Amanda."

Mr Acedo further outlined, "I wouldn't describe Allan's behaviour as physically intimidating. I could see as soon as Allan did it, he knew it was the wrong thing to do and seemed apologetic. To me, Allen was frustrated and let steam off. I didn't think it was physical intimidation but if it was directed at me, I may have felt differently. Just after the incident, Amanda stormed out of the room crying. After the meeting, I saw Amanda in the corridor being consoled by another staff member, Kylie Rika. I had no further involvement following this."

Further, "I am not aware of any issues between Amanda and Allan in the past. Apart from this incident, I have never observed any behaviour from Allan that I would describe as aggressive or inappropriate. I would describe Allan's communication style as generally professional. He can be set in his ways at times."

# Allison Lloyd states:

Allison Lloyd is Reporting Scientist in the Reporting Team. At interview Ms Lloyd outlined, "I was present at the management team meeting on 9 June 2016. I was sitting directly opposite from both Allan McNevin and Amanda Reeves at this meeting. I was there observing as my supervisor hadn't left yet and I was asked to attend on that date. The meeting was progressing as normal until it reached the section about projects."

Further, "When we got to project #181 about sperm not being seen in case work, there was discussion about how it was progressing. Allan had talked about how the project plan had written and was currently with management for feedback. Allan was sitting next to Amanda, Amanda said she had some misgivings about the project plan and some of the content of the project plan were not pertinent."

Ms Lloyd further outlined, "I remember Amanda said something and Allan became very defensive. Allan raised his voice his voice quite a bit, probably closer to a yell. He was moving his arms up and down and I think he banged on the table several times. He turned towards Amanda. He said something like, that Amanda always only had one concern and he had addressed it and she couldn't move on. I can't remember the exact words. Deb Whelan, the acting managing scientist and Justin Howes, who is in charge of the reporting and intelligence







teams had to call several times for Allan to stop and clam down. Deb Whelan said, 'Ok we'll leave this issue for the moment and move on.'"

Further, "The meeting went back to normal and Amanda left the meeting for most of the meeting. Allan sat in the meeting with his head down looking at the table. Amanda then returned to the meeting near the end and it was apparent that she had been crying."

Ms Lloyd further outlined, "In relation to Allan's behaviour at the meeting, I think he was frustrated. His actions were more expressing frustration than being physically intimidating. Having said that, if I was sitting next him and he was speaking to me, I could see that it could be physically intimidating. I don't think it was his intention though. Amanda can be quite forceful. But on this occasion, I thought Amanda was just asking questions and expressing her view about some matters weren't being addressed. I'm not sure what had happened at other management team meetings. I know that Amanda has said in the past that Allan and her don't see eye to eye and they would never be best friends. Amanda thought that Allan didn't like her and she does not like Allan. Nothing like this before though."

#### **Consideration of Evidence**

The evidence is consistent that on 9 June 2016, at the Management team meeting, Allan McNevin provided the meeting with an update on Project #181. During this update, Amanda Reeves sought information from Mr McNevin. The conversation became heated and Mr McNevin raised his voice at Ms Reeves. Mr McNevin said something like, "I'm not stupid, I know the risks." The exact wording cannot be established however, there is a general consensus in the evidence that the words were along these lines.

There is inconsistent evidence about the volume of Mr McNevin's voice. Evidence varies from a 'raised voice' to Mr McNevin 'yelled' at Ms Reeves. Mr McNevin described that he shouted at Ms Reeves. By Mr McNevin's own admission, his voice was more than raised and that he shouted at Ms Reeves.

There are also inconsistencies in the evidence as to whether Mr McNevin hit the table with his hands when he was shouting at Ms Reeves. Mr McNevin stated that he pushed himself away from the table. Ms Reeves stated that she "saw his arms raised and then brought down with a noticeable and audible impact. In the same movement he pushed himself back from the table and turned toward me whilst yelling."

The evidence ranges from corroboration of Mr McNevin's evidence that he pushed himself from the table to that Mr McNevin was banging his fists on the table. The inconsistencies in the evidence do not allow for a definite finding to be made as to the force of Mr McNevin's hands making contact with the table. There is, however, consistent evidence to establish that Mr McNevin made contact with the table and pushed himself back from the table when he shouted at Ms Reeves.

Mr McNevin was sitting next to Ms Reeves during the meeting. Mr McNevin outlined that he turned towards Ms Reeves when he shouted at her.

The Macquarie Dictionary defines 'intimidating' as 'threatening.' This means that there must be a threatening element to Mr McNevin's conduct. Further, Ms Reeves' evidence and concerns refer to the physicality of Mr McNevin's conduct. Hence, witnesses were requested to provide their views to whether they considered Mr McNevin's conduct to be 'physically intimidating.'

The majority of witnesses outlined that they did not consider Mr McNevin's conduct to be physically intimidating. Most witnesses outlined that Mr McNevin's conduct was that of frustration rather than intimidation. Further, it is the view of the investigator that Mr McNevin's physical stature and/or training in martial arts is relevant to Ms Reeves' perception of Mr McNevin but not relevant to Mr McNevin's actions on the day.







The evidence is also mixed to whether Ms Reeves was being reasonable in her questioning towards Mr McNevin. The evidence ranged from Ms Reeves just asking questions of Mr McNevin to Ms Reeves being confrontational, very emotional, intense and disproportionate. It is difficult to establish whether Ms Reeves was being unnecessarily provocative towards Mr McNevin.

Whether, Ms Reeves was being unreasonable or not in her questioning of Mr McNevin, it was not appropriate for Mr McNevin to shout at Ms Reeves. However, there is insufficient evidence to establish that Mr McNevin 'intimidated' Ms Reeves by his actions. The evidence supports that Mr McNevin reacted out of frustration rather than that of intimidating Ms Reeves. While Ms Reeves' testimony is that she felt physically and emotionally intimidated, there was no evidence presented that Mr McNevin's conduct was threatening towards Ms Reeves. Considering the evidence presented by witnesses of the incident the investigator is of the view that a reasonable person would not consider Mr McNevin's conduct as intimidating.

While Mr McNevin's behaviour was not appropriate for the workplace, the evidence is that it was a 'one-off' incident which was totally out of character. He has admitted his behaviour was unacceptable, demonstrated remorse, apologised on three occasions and has been counselled about this behaviour by his manager. It is the view of the investigator that commencing disciplinary action against Mr McNevin for this matter is not warranted.

In relation to whether Mr McNevin's behaviour was an 'unacceptable escalation of his existing behaviours', the majority of witnesses outline that Mr McNevin is usually a 'laid back' person and that this behaviour was out of character.

There are varying opinions in relation to Mr McNevin's communication and behaviours. Ms Rika outlined she believed that while Mr McNevin's communication style is very relaxed at management team meetings he appears very nonchalant. Further, it was Ms Rika's perception of the way he communicates with Ms Reeves, that he can be dismissive of what Ms Reeves is saying and is a hassle. Contrary to this, Ms Johnstone outlined that it was very out of character for Mr McNevin to act in this manner and that he is usually very level. Further, that Mr McNevin comes up with 'out of the box ideas' and tends to talk a lot but rarely shows any emotion. Ms Johnstone further stated that "Allan is really friendly, his whole team loves him. He has managed a number of staff over the years and does a really good job of it."

While both Ms Reeves and Mr McNevin allude to some communication difficulties between them in the past, there is insufficient evidence to support that there are on-going issues between them. Ms Reeves and Ms Rika refer to ongoing tension between Ms Reeves and Mr McNevin, there was no evidence presented that referred to any particular incidents. The investigator is of the view that the 'ongoing tension' referred to is more about Ms Reeves' perception that Mr McNevin doesn't value her opinions and is dismissive of her rather than any specific incidents or confrontation. This was also supported by Ms Rika in her evidence which outlined that Ms Reeves "feels others, such as Allan are being dismissive and not placing importance of what she says."

There is insufficient evidence to support the part of the allegation that Mr McNevin's conduct was an unacceptable escalation of his existing behaviours.

# **Findings**

In relation to the allegation that Mr McNevin's response to feedback provided by Ms Reeves in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours is **not substantiated**. However, there is sufficient evidence, including Mr McNevin's admission that he shouted at Ms Reeves which is not consistent with the *Code of Conduct for the Queensland Public Service section 1.5 'Demonstrate a high standard of workplace behaviour and personal conduct.* 







# Allegation 2

Amanda Reeves alleges that Allan McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships.

## **FINDING: Not Substantiated**

#### Overview

In an email to Paula Brisotto, titled 'Meeting followup' dated 16 August 2016 (AR29), Ms Reeves outlined that she was withdrawing from the facilitated meeting that had been previously agreed to in which Jade Franklin, Manager Human Resources and Business Relationships, HSQ, was to facilitate. One of the issues outlined by Ms Reeves for the withdrawal was the that she perceived "Allan's determination to take and immovably maintain a 'stance', an associated unwillingness to be open to feedback or to consider making adjustments to his attitude or behaviours in the interests of improving workplace relationships/the working environment/work outcomes."

Ms Reeves further elaborated in her interview in relation to this concern. Ms Reeves did not refer to any specific incidents prior to the incident on 9 June 2016, but rather referred to Mr McNevin's conduct in general terms. Mr McNevin's alleged conduct both prior the incident on 9 June 2016 and post the incident was taken into account. The issue related to Mr McNevin's alleged 'changes to standard wording' in Ms Reeves' email is dealt with in Allegation 3.

The witnesses interviewed were able to provide direct evidence in relation to dealings with Mr McNevin in relation to Ms Reeves both prior and post the incident on 9 June 2016. While other witnesses were questioned about Mr McNevin in relation to this allegation, they were not able to provide direct evidence for this allegation. Notes were taken of the meetings by Ms Whelan, Ms Reeves, Ms Brisotto and Ms Caunt and were considered by the investigator. Ms Caunt was Ms Reeves' support person and provided Ms Reeves with notes which Ms Reeves provided to the investigator. These notes are referred to and attached to the individual's statements.

# **Available Evidence**

#### **Amanda Reeves**

In Ms Reeves email to Ms Brisotto dated 16 August 2016, Ms Reeves outlined the following, "I perceive issue 1 being Allan's apparent determination to take and immovably maintain 'a stance'. An associated unwillingness to be open to feedback or to consider making adjustments to his attitudes or behaviours in the interests of improving working relationships/the working environment/work outcomes.

- I have for many years now, consistently had conversations with my line manager about how difficult I find it to 'be heard' by Allan during the provision of project feedback and management meetings.
- I believe that informal chats have been held with Allan by a number of people about the negative impact the lack of eye contact/pulling a hoodie over his head, clicking of pens, fiddling with objects, slouching, sighing, using loaded language like 'semantics' and 'alarmist' when responding to feedback can have on effective communication between people, but I have seen nothing to suggest he has taken this feedback on board in such a way that the behaviours are modified.
- I have previously tried to attempt to try and achieve professional communication with







Allan – when I referred to this in our recent meeting Allan's response was 'yes you have always had an issue with me, but I am who I am – I can't help how your perceive me."

At interview, Ms Reeves outlined, "While we have equal standing at the management table, I feel that unless my opinion gels with Allan's he dismisses me out of hand. I have raised concerns with my line manager Justin Howes (HP6 Forensic Reporting & Intelligence Team - FRIT) about this in the past. As a result of this, I met with Allan to address the issues. This meeting was a couple of years ago. Allan seemed to listen to me at this meeting but in my opinion his behaviour did not change following this. There have been other attempts to address this through my line manager. I met with Allan on 8 August this year to attempt to resolve this issue. Allan's response was that 'you have always had a problem with me, I can't help how you perceive me, I am who I am'. My interpretation of that was Allan wasn't listening and he wasn't prepared to compromise."

Ms Reeves further elaborated on the meeting on 8 August 2016, "Allan specifically restricted his apology to having just raised his voice, and he did not want to acknowledge the distress his actions caused me. I mentioned that I was frightened and it could be considered assault, and if it happened again I would call the police. I asked if he could assure me it wouldn't happen again. Allan said that he couldn't control how I interpret his actions."

Further, "I stated that Allan was not willing to take on board my position and had no respect for me. He reiterated he shouldn't raise his voice. I said it was an escalation of existing behaviour and I needed it to stop. I said I deserved respect and should be able to raise issues. He said he was frustrated that I raised the same issue again. I said we have had issues before but you don't see this. He said you have issues with me. I said he didn't seem at all apologetic and his original apology seemed flippant. He said he tries to keep communication relaxed and intended to follow it up. He said he understood I was upset but he wasn't sure what I expected from him. He said that I say he dislikes me but I don't know how he feels inside. I replied that I assess him on his actions, and he replied that he can't control my emotional response to him."

#### Allan McNevin states:

At interview, Mr McNevin outlined, "Amanda has a strong personality. She can often go straight on the front foot about a matter. She can be quite persistent when she wants to put a point forward. It can be very frustrating in that Amanda can labour the same point over and over again without bringing new information to the discussion."

Further, "I think over the years, I have managed my discussions with Amanda very well. I have been commended on this at times by others on the management team. I have worked on different strategies to deal with this. Sometimes, I will disengage when I can see the conversation is going nowhere. Sometimes I will sit back from the table and doodle in my book rather than engage in the conversation. I have been told that Amanda is intimidated by my scientific knowledge and sometimes will try to engage on a matter where she doesn't have the full knowledge about the matter and will try and argue about it. I find that difficult. I don't try to ignore her but sometimes it's difficult to engage."

Mr McNevin outlined his recollection of the meeting on 8 August 2016, "I met with Amanda about three days later, Amanda brought Emma Caunt with her and I asked Kirsten as she had been in the meeting and I didn't want to involve others. We met, I apologised again. Once again she said she accepted my apology. She then started ripping into me saying things like her husband was ill and it was unfair of me to do that. She said she felt unsafe and was standing up and being quite animated while I was sitting down. She said what I did was borderline assault and she would seek legal action if it happened again."

Further, "I did my best to be quiet through this. I acknowledged I did the wrong thing. I explained







that I felt she was treating me like I was stupid. She said it was her right to raise risks. She brought up my karate training and how it was borderline assault. I tried to explain that my training was not relevant to the matter."

Mr McNevin further outlined, "I tried to say that she had interpreted my actions in one way and she couldn't understand how I could interpret her speech and actions and feel frustrated. I asked her what she wanted me to do and she said she just wanted to be treated with respect and not yelled at. I asked her if there was anything she was willing to change and she said no, as she always acted professionally and didn't need to change."

Further, "The meeting ended there. The meeting went on for about 15 to 30 minutes. Emma or Kristen may have taken notes about the length and the detail of the meeting. I felt like I have done everything I could to rectify what happened in the management meeting. I offered to make amends but Amanda didn't seem to want to accept it. I did ask Amanda if she would like mediation but Amanda said she had a bad experience with mediation."

#### Paula Brisotto states:

Paula Brisotto is the Team Leader of the Evidence Recovery and Quality Team. Ms Brisotto is Mr McNevin's line manager. Ms Brisotto was on leave at the time of the incident of 9 June 2016. Kirsten Scott relieved in Ms Brisotto's position while on leave. At interview Ms Brisotto outlined, "On 5 August 2016, Deb Whelan had a meeting with Allan and me. I was there as Allan's supervisor. Deb indicated that she took advice from HR. Allan was to make a more formal apology to Amanda but wasn't required to apologise to the other participants at the meeting unless they requested one."

Further, "Allan also asked whether Amanda would be required to apologise for her email. Deb responded that there was no advice from HR that this was required to happen. Deb also warned Allan that if it happened again, then there would be more formal action. On that same day, I believe the apology email was sent from Allan to Amanda. A meeting was also organised for the two of them for the 8 August."

Ms Brisotto further outlined, "The meeting did occur on the 8 August between Amanda and Allan and each brought a support person. I wasn't present at the meeting and wasn't at work that day. I returned the next day and was advised by the participants that it didn't go well. Later that day I met with Amanda and her support person Emma Caunt. Emma had to leave half way through."

Further, "I was advised the meeting didn't go well. Amanda advised that she didn't receive a sincere apology and only apologised for the shouting and not the physical actions. Amanda also said Allan appeared be only apologising because he had to and he appeared not to care because he was not reactive. These are the views expressed by Amanda."

Ms Brisotto continued, "I recall talking to Allan about his body language as he can appear fidgety. He said that was his coping mechanism. I suggested that he can look distracted and gave him some advice about how to address this."

#### **Kirsten Scott states:**

At interview Ms Scott outlined, "From my point of view, Allan acknowledged he had made a mistake and acknowledged this. Apart from losing his cool, Allan couldn't have acted more appropriate to correct the matter. Amanda didn't appear to be ready to resolve the matter at that stage."

In relation to the meeting on 8 August 2016, Ms Scott outlined, "I didn't have further involvement until a fair bit later when I was Allan's support person in a mediation type meeting with Amanda. I didn't take notes of the meeting. I don't remember the specific words or



conversations of that meeting. What I recall is that it wasn't very constructive. From my point of view, Allan was trying to reach some middle ground but Amanda wasn't trying to resolve the issue. The meeting didn't last very long."

#### **Deborah Whelan**

At interview Ms Whelan outlined the following, "After the meeting ended (9 June 2016), I stayed behind with Justine to talk to Allan about the incident. I said to Allan that his behaviour was inappropriate and he needed to apologise. Allan accepted his behaviour was inappropriate and was willing to make an apology to Amanda. Allan indicated that he would apologise immediately and we finished the meeting."

Further, "On 10 June I met with both Allan and Amanda separately. The meeting with Allan was straightforward. He again admitted he did the wrong thing but was frustrated by Amanda saying the same thing over and over again. Allan was also unhappy about the content of Amanda's email and was concerned she was aiming to use the incident against Allan in the future. I made a note of the meeting (DW1)."

Ms Whelan further outlined, "I had a further meeting with Allan on 5 August 2016. I asked Allan's supervisor Paula Brisotto to attend as my secondment was coming to an end. I made notes of this meeting (DW6). Allan outlined that he did apologise on the day but was willing to make a more formal apology as the first one appeared flippant. Allan wanted to know why Amanda wasn't apologising for her email. Allan acknowledged his behaviour was inappropriate and if it was repeated there would be consequences. Allan also indicated that he would apologise to other meeting attendees if they raised concerns with him. The outcome was that we were managing Allan's behaviours so there was no need for any written assurances from Allan. Also were intending to address management behaviours."

# **Consideration of Evidence**

The witnesses interviewed in relation this allegation were interviewed as they were in attendance at meetings with Ms Reeves and Mr McNevin following the incident and were able to provide direct evidence of Mr McNevin's and Ms Reeves conduct at these meetings. Written notes of the meetings prepared by the witnesses are attached to their respective statements and were considered by the investigator.

This allegation centres very much on Ms Reeves' perception of Mr McNevin's behaviour and his responses to Ms Reeves and Ms Reeves' expectations of how she believed Mr McNevin should behave. In the email of 16 August 2016 to Ms Brisotto, Ms Reeves outlined three points this allegation is based on. The first two dot points refer to perceptions of Mr McNevin's general communication techniques over a period of time prior to the incident. The third dot point refers to Ms Reeves raising the concern with Mr McNevin at a meeting on 8 August 2016.

The first dot point outlines that Ms Reeves has consistently had conversations about how difficult she finds it to be 'heard' by Mr McNevin during the provision of project feedback and management meetings. The second dot point refers to Ms Reeves' belief that a number of informal chats have occurred with Mr McNevin about his actions and language and the effect these can have on positive communication and Ms Reeves has seen nothing to suggest Mr McNevin has taken this feedback on board and modified his behaviour.

Ms Reeves refers to issues raised with Mr Howes, Ms Reeves' supervisor. At interview Ms Reeves outlined that these conversations resulted in a meeting between Ms Reeves and Mr McNevin 'a couple of years ago'. Further that there has been 'other attempts to address this through her line manager'.







Ms Brisotto outlined that she has had discussions with Mr McNevin about his body language and how he can appear fidgety. Ms Brisotto stated Mr McNevin responded by saying that this was a coping mechanism.

Ms Whelan's evidence is that Mr McNevin was remorseful, willing to make amends by apologising and also acknowledged his behaviour was inappropriate and if repeated, there would be consequences. The investigator is of the view that Mr McNevin is, in fact, willing to accept feedback and adjust his behaviour despite this being a 'one off', out of character incident.

Mr McNevin at interview outlined that Ms Reeves has a strong personality, persistent in labouring points without raising new information and starts conversations on the 'front foot'. Further that he disengages when conversations are going nowhere.

Following consideration of the evidence presented, it is the view of the investigator that Mr McNevin and Ms Reeves are both confident, experienced professionals who have different communication styles. Mr McNevin outlined that he prefers factual engagement and when conversations veer away from this, he disengages. Further, a number of interviewees outlined that is laid back whereas Ms Reeves can engage in a forceful and confrontational way.

In relation to the meeting of 8 August 2016, both parties concede that it wasn't successful. The evidence is that Mr McNevin offered an apology, his third since the incident on 9 June 2016, but Ms Reeves was not satisfied with this. In her own words, "Allan specifically restricted his apology to having just raised his voice, and he did not want to acknowledge the distress his actions caused me." Ms Reeves went on to say that she considered Mr McNevin's behaviour as assault and that if it occurred again she would call the police.

It is the view of the investigator that this escalation of the incident by Ms Reeves to that of a criminal matter placed Mr McNevin in a very difficult position in that if he apologised to the satisfaction of Ms Reeves he is admitting that he 'assaulted' Ms Reeves otherwise he is refusing to acknowledge his behaviours and make the perceived adjustments. The escalation of the interpretation of events by Ms Reeves since the incident makes it very difficult for Mr McNevin to satisfy what Ms Reeves expects in an apology. It is the view of the investigator that Mr McNevin has acknowledged his mistake in a genuine way. Whether Ms Reeves accepts the apology is beyond Mr McNevin's control.

Mr McNevin is correct when he states he cannot control what perceptions Ms Reeves forms from Mr McNevin's communication style. Further, the meeting of 8 August 2016 demonstrated that unless Mr McNevin accepts Ms Reeves' perception of his behaviour and communication style, his response is unacceptable to Ms Reeves.

Mr McNevin's communication style is by no means perfect and could be improved by Mr McNevin being assisted to adopt techniques to redirect conversations when they are becoming 'bogged down.' Having said that, Ms Reeves must also reflect on her own communication style and techniques and how it has contributed to the difficulty between her and Mr McNevin. For their relationship and communication to improve, both Ms Reeves and Mr McNevin need to be willing to accept their shortcomings and be accountable for their communication styles and work to improve this.

It is unfair to place the onus solely on Mr McNevin for the difficulties over the years. Further, other than Ms Reeves' perception, there is no evidence to support that Mr McNevin has been unwilling to be open to feedback or to consider making adjustments in his attitudes or behaviours in the interests of improving workplace relationships.







## **Findings**

The allegation that Mr McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships is **not substantiated**.

# Allegation 3

Amanda Reeves alleges that an email from Justin Howes, Team Leader to all reporting staff on the 5 of August 2016 regarding standardised statement wording was specifically directed at Mr McNevin.

#### FINDING: Not Substantiated

#### Overview

In an email to Paula Brisotto, titled 'Meeting follow up' dated 16 August 2016 (AR29), Ms Reeves outlined that she was withdrawing from the facilitated meeting that had been previously agreed to in which Jade Franklin, Manager Human Resources and Business Relationships, HSQ, was to facilitate. One of the issues outlined by Ms Reeves for the withdrawal was the that she perceived "Allan's determination to take and immovably maintain a 'stance', an associated unwillingness to be open to feedback or to consider making adjustments to his attitude or behaviours in the interests of improving workplace relationships/the working environment/work outcomes."

In this email, Ms Reeves cited as an example Mr McNevin's alleged practice of changing standardised wording for statements. Ms Reeves further alleged that this practice prompted Justin Howes to remind all reporting staff to use the standardised wording but that the email from Mr Howes was specifically directed at Mr McNevin.

As this allegation refers to Mr Howes' email, Mr Howes was the only person in a position to provide direct evidence about this matter.

## **Available Evidence**

## **Amanda Reeves:**

In the email to Ms Brisotto, Ms Reeves outlined the following:-

"I am also aware that I am not the only person who struggles with this behaviour — as the line manager responsible for allocating casework, I have been advised several times that staff are not happy, sometimes unwilling, to take Allan's casework for review, because he changes standard wording (so that he can 'tell a story'), and when they try to broach with him that the statement is meant to be a standardised vehicle for translating technical jargon into simple terms for the target lay audience, he staunchly defends his position, despite being isolated in that position."

"Staff report that they find this process difficult, and they are concerned about having to potentially defend changed wording in court that they themselves don't easily understand and could potentially be incorrect. This has required yet another email, sent to all reporting scientists, on 5<sup>th</sup> August —

Hi all







A few instances of late have been brought to my attention where the collective agreement on statement wording hasn't been used. This wording for STRmix statements had the opportunity for input from all reporting scientists in meetings in 2013 and as an outcome, the wording was standardised and put into the 17119 SOP. There were many reasons for this, and apart from an important point of standardisation, it was to help any scientist to pick up any statement at any time and be comfortable with the wording, and also to help reviewers efficiently perform their task with minimal disagreement.

Can I please ask that we stick to the standard wording in the interests of the above as we need to put all our efforts/time into getting the large amount of work to our clients.

Thanks

JAH."

#### **Justin Howes:**

During the investigation process, Mr Howes outlined in an email response to the investigator that he did send the email referred to by Ms Reeves to all staff competent in court reporting (or in training as a court reporting scientist).

Mr Howes outlines that the purpose of the email was "to ensure all staff are following standardised wording in statements. I write these general emails when more than one person, and more than one instance has occurred, where they appear to be drifting from the standard approach. If there are instances that relate to one person, and especially more than one time, then an email wouldn't be written rather a discussion would need to occur with the person."

Further, "The email was not specifically directed at one person. These emails are an attempt to correct more than one person who may have started to drift from the standard approach, and to remind all reporting scientists to the benefits of standard wording."

Mr Howes further outlined that "it was not unusual for these general emails to be sent regarding a range of processes/practices."

## **Consideration of Evidence**

Ms Reeves based the allegation on her perception that the purpose of the email was to correct Mr McNevin's behaviour in relation to his alleged deviation from standardised wording. Mr Howes is clear that the email was not specifically directed at Mr McNevin but rather a general reminder to all staff. Mr Howes further outlined that if there were instances that related to one person, that this wouldn't be undertaken via an email but rather a discussion would occur with that person.

Ms Reeves did not provide further evidence than this assertion in the email.

If Mr McNevin's alleged deviation from the standard wording is of concern, it is best dealt with by Mr McNevin's line manager, Ms Brisotto, or by Mr Howes directly with Mr McNevin.

# **Findings**

Mr Howes has clearly indicated the email referred to in this allegation was not directed at any individual therefore Allegation 3 is **not substantiated**.







# Allegation 4

Amanda Reeves alleges Allan McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide and caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so.

#### **FINDING: Not Substantiated**

#### Overview

This allegation is that Ms Reeves alleges Mr McNevin deliberately delayed and/or obstructed the progress of project #181 which potentially put the organisation at risk. In Ms Reeves' email to Ms Brisotto on 16 August 2016 (AR29) she outlines that as of 8 August 2016, Mr McNevin outlined in an email that the process had been changed. Ms Reeves outlined in that email this vindicated the validity of her feedback. Further at interview Ms Reeves outlined that the risk had been mitigated. (Please note this report does not examine the scientific factors of Project #181.)

Interviewees were asked questions in relation to their knowledge of Project #181, their views on Mr McNevin's management of it and other general concerns about the project. Only direct and relevant evidence provided by the interviewees was considered and incorporated into the report.

# **Available Evidence**

## **Amanda Reeves states:**

At interview Ms Reeves outlined, "We seem to have different opinions about the risk and the urgency of the problem. From my perspective, I had raised the issues at least on 4 March 2016, if not before. I have an email that demonstrates. There are a series of three emails (AR2 i, ii, iii). The first email from Jacqui Wilson describes the issues. The staff member suggests the issue is with preparation of the slide itself and suggests an investigation. My response is directed to Justin Howes with a suggestion that an investigation is warranted which could be widened if required. I offered support for this."

Further, "The third email is from Justin Howes to Jacqui Wilson and myself who said, "Great timing in catching Luke and I together on this! We are also together on the fact that these two reads being vastly different is worth looking into further. Thanks for raising your concern – if that wasn't done, there wouldn't be anything we could do to find out and action this outside of audit schedules. Good work and we will follow things up here." At the time of this email, Luke Ryan was the acting HP6 (Evidence Recovery Analytical & Quality Team - ERQ)."

Ms Reeves further outlined, "I was told when I offered my staff member's time because the matter is within Allan's team responsibility, that team would deal with it (ER). I believed that it had been escalated to the relevant HP6 (ERQ). I have an email dated 6 May 2016 from Justin Howes to Jacqui Wilson and copied to me (AR3). This email reflects that Justin had followed up with ERQ on the lack of progress with this investigation. This was some two months after the issue was raised. I was receiving a number of enquiries from staff about this matter. I received an email, 9 May, from a staff member about this matter and I advised that he forward the example to Justin Howes, Kirsten Scott, Kylie Rika and myself.(AR4)"

Further, "On 11 May, I came across a number of staff members from the Reporting Team congregating and discussing concerns about the lack of action. I said to them if they were going to use their time to discuss the issues, I wanted the issues documented and possible solutions put forward. It is worth noting that whilst the Reporting



scientists (HP4) physically don't prepare the slides, they are trained in the process, and in the interpretation of the slides and associated other evidence/tests performed by ERQ and Analytical scientists (HP3), in order to attend court and give expert/opinion evidence. Following my discussion with the staff, I received an email on 11 May from Anna Lemalu copied to the participants (AR5). The email provides suggestions for the slide investigation. It is important to note the emails conclusion, which states "The major overarching concerns of this issue are the fact that in certain circumstances we may not have sent samples for DNA profiling at all (micro, AP and PSA neg) and have therefore missed evidence. Also, occasionally we are asked in court specifically about the number of sperm seen in a sample – if we know that this number is unreliable, how happy will reporters be to quote numbers?" That email was copied into a new email sent by Justin to Allan, copied to Kirsten Scott (acting ERQ HP6) on 12 May (AR6)."

Further, "We had a Management meeting on the 12 May. We discussed the issue raised in the email. It was the first time this matter was discussed at the Management meeting, as 'New Business'. In my opinion, the minutes do not fully document the conversation held. I was concerned, as were others in my team, that we needed to check the initial slide so we weren't missing anything as we were at risk of not detecting evidence, which is the core business of the team."

Ms Reeves further outlined, "On 27 May we had a Management meeting where Allan and I had a robust discussion about this matter. Although Allan and I were not in agreement about the urgency of the risk and the scope of the project, I didn't feel intimidated in this discussion. I believe Kylie Rika shared the same concerns as me. I held the position that the immediate risk needed to be stemmed, and once that was addressed, as long as the process for making the ER slides was investigated, the project scope could include whatever else Allan wanted. The minutes did not accurately capture this conversation (AR8)."

Ms Reeves further stated, "There are two levels of risk as I see it. The first category is where the microscopy is negative when there is truly sperm there but the seminal fluid component is not detectable. In this situation there is no 'safety net'. It either gets missed completely, or it goes though an extraction type that doesn't allow for check (differential) slides. The aim of differential lysis extraction is to separate female (epithelial) and male (spermatozoa) cells. The risk here is that if sperm isn't detected at the initial stage but is present in small numbers, the sample may not be sent for differential lysis extraction, and the male component could be 'swamped out' by the female component in a mixture."

Further, "The second level is where the microscopic slides are negative but there is truly sperm there, but the seminal fluid component is detected. The detection of the seminal fluid causes the sample to progress through differential lysis extraction, during which a second set of slides are made (diff/check slides). This second set of slides provides the 'safety net', but they are not routinely examined – they have to be specifically requested. I have copy of the workflow which may assist (AR9). The risks appear to be due to a deficiency in the microscopy process, which is at the beginning of the workflow, the results of which direct the progression of the sample through the remainder of the workflow. There are several cases where this occurred in relation to the second level risk. I'm not aware if we have specifically retrospectively checked for cases exposed to the first level risk. I'm not sure that we will be able to easily identify them, at least not until the microscopy process is fixed."

Ms Reeves further outlined, "The initial request for the project was made on the 2 June 2016 (AR10). On 19 July I have two emails that indicate that there was still concern from the Reporting group about the slides issue. I forwarded the email that Kylie sent to her team to Justin, where I give an example of the issue and expressed that we needed this sorted ASAP. I outlined that I was very concerned and asked for it to be followed up with priority. I received an email from Jacqui Wilson on 20 July giving an example from 2015 where the slides indicate the problem. I responded to Jacqui — "Thanks Jacqui. Justin has assured me that he has followed up with Paula, who will be following up with Allan. Unfortunately there have been no timeframes given yet, but I have asked again that this be given urgent attention"



Ms Reeves concluded the interview with the following statements, "I was advised during the interview that one of the documents provided to the investigator by way of background was the Procedure for Change Management in Forensic DNA Analysis Standard Operating Procedure (SOP). This document is a guide for controlling change to processes in the lab — "changes within Forensic DNA Analysis have the potential to impact on our clients, on stakeholders (internal/external to FSS) and may impact on compliance with NATA. As such changes which occur with Forensic DNA Analysis must be carefully considered and documented. There are a number of types of changes that may occur within Forensic DNA Analysis; for the purpose of documentation - these are classified into five types: administrative change, IT/LIMS change, minor project, major project, and external projects."

Additionally, "I provide Section 4.11 Corrective Action, in the AS ISO/IEC 17025 Australian Standard "General Requirements for the Competence of Testing and Calibration Laboratories", against which we are assessed for compliance by NATA. (AR30) This standard provides that a problem with technical operations of the laboratory may be identified from staff observations, and should have a root cause analysis/investigation undertaken, and then corrective measures implemented. I provide The Procedure for Quality Practice in Forensic DNA Analysis SOP and the first two pages of the Investigating Adverse Events in Forensic DNA Analysis SOP — I have not provided the entire document, as only the first two pages are relevant to this issue, but can do so upon request. These documents should be considered alongside the Change Management SOP."

Further, "With reference to these documents and this issue - I escalated a potential deficiency with a critical process to the relevant senior staff in March 2016. At this point, an investigation/root cause analysis and risk assessment should have been conducted, followed by corrective action in accordance with our SOPs and the relevant Standard. This was not carried out, in my opinion because the risk was possibly not fully understood and was being minimised by Allan, and thus the issue was instead treated as a non-time-sensitive project proposal through the change management process. I am of the belief that this issue should have been managed in the first instance as an adverse event."

## Allan McNevin states:

At interview Mr McNevin outlined the following, "There was an issue that arose in relation to testing for spermatozoa and the difference of evidence recovery and the differential slide. There were concerns raised that that there was risk for that threatened the collection of evidence. While I acknowledged this might be a risk, I believe we needed to look at the evidence and the risk and agreed it needed to be investigated. We had some discussions at two previous management meetings. I agreed that it could be a risk and it needed to be investigated. While I like to approach things with hard evidence, Amanda would often make comments like, "If we miss this than a guilty person walks free." This is often how Amanda will approach things, but I don't find it helpful.

## **Kirsten Scott states:**

At interview Ms Scott outlined, "In relation to project #181, I wasn't aware of discussions about it until I relived in the team leader role. As soon as I became aware of the matter, Allan and I addressed it straight away. I believe Allan understood the risks of the issue. Allan may have had a different view on how to approach the matter which didn't mean he took it any less seriously. The laboratory has never collected data in relation to this issue previously so it was difficult to say whether it was risk or not. If we had the data, we could have gone directly to addressing the issue but we didn't have the data. We have implemented a temporary solution to address the risk but it isn't a long-term solution. It is a way to address the risk if it is in fact an issue."



#### Adrian Pippa states:

Adrian Pippa is a Reporting Scientist for the DNA Analysis Unit. At interview Mr Pippia outlined, "In relation to Project #181, I was asked by Amanda to provide input and suggest some experiments that would be appropriate. I assume, Amanda acted on these and progressed them. I had to meet Allan McNevin to discuss some aspects of the project plan. I believe Allan took my suggestions on board and adjusted the project plan."

Further, "I think the progress of Project #181 has been quite slow considering it's importance as it has identified a risk that I believe has been present for a number of years. I am not sure of the reason for the delay, I believe it may because other matters have been prioritised over it by the management team. I am not aware of any individuals who may have deliberately delayed or been obstructive in relation to the project."

Mr Pippa further outlined, "I think there is a tendency in the laboratory to over-complicate matters which can contribute to blown out timeframes. Having said that I think we have really good skills to resolve the issues but we do tend to overcomplicate matters. In relation to project needs, Project #181 could have been done in parts where the spermatozoa detection (microscopic aspect) could have been done first and then the enzymatic testing could have followed."

# Jacqui Wilson states:

Jacqui Wilson is a Reporting Scientist for the DNA Analysis Unit. AT interview Ms Wilson outlined, "In relation to Project #181, as I come from a background of being in the sexual assault team, I am probably more aware of the issues of concern. I have been concerned for the last couple of years about a possible potential issue with the slides and possibly evidence being missed."

Further, "I have raised the concerns with the team managers and then left them with them to manage. I not aware of evidence being missed but more that there was potential for be missed. I am aware that the project #181 was established to examine the issue. I understand that there needs to be gathering of information or more examples to move forward with that. I don't believe that there have been any deliberate roadblocks to addressing the issue. Since then, there has been a workaround implemented in the meantime to address the issue. We are very busy department and these sort of issues take time to address.

#### Valerie Caldwell states:

Valerie Caldwell is a scientist in the Evidence Recovery Team. At interview Ms Caldwell outlined, "Project #181 is good example of communication issues. Initially, the two teams weren't communicating and it was difficult to understand the issues. Since then, communication has improved. In my role I do the testing of the slides, and the rechecking at the end. The work around has addressed the concerns but it is has increased our workloads. I will be interesting to see if the project identifies what can be done to address this issues."

Further, "In my view, Allan has acted appropriately in addressing the concerns raised in relation to the issues that commenced project #181. He copped flack from us in relation changing the testing but he also copped the flack from the other team in relation to the issue. He was in a very difficult position. I find that the major thing is that sometimes we rush to solutions when the problem isn't really understood which leads to having fix issues that haven't been considered. Also we are at the forefront of our field which leads to issues where we might be having teething problems."







#### **Thomas Nurthen states:**

Thomas Nurthen is a Reporting Scientist for the Forensic DNA Analysis Team. At interview Mr Nurthen outlined, "I have had no direct involvement in project #181 but I had involvement in trying to get something done in leading up to the project. As a reporting group, we identified there was an issue and as a group we met in May 2016. We knew of problems prior to this. A solution wasn't implemented until August. In my view we had enough information to act on it in May. I think when an issue is identified, unless it is a burning issue, it doesn't get addressed as quick as it should. I think the delay was because of this."

#### **Consideration of Evidence**

On consideration of the available evidence, the investigator is of the view that Project #181 was viewed differently by the different teams within the Forensic DNA Analysis team. Generally, the Reporting team, led by Ms Reeves viewed the issues as something where a solution could be reached reasonably quickly whereas the Evidence Recovery team, led by Mr McNevin believed more scientific analysis was required before reaching a solution.

Ms Reeves outlined in her evidence that the issue was first discussed at a management meeting on 12 May 2016. Further, it was discussed on 27 May 2016 where Ms Reeves outlined, *Allan and I were not in agreement about the urgency of the risk and the scope of the project.*" Further, Ms Reeves stated that the initial request for the project was made on 2 June 2016. Evidence was presented that a temporary solution, which addressed the problem was implemented on 8 August 2016.

Mr McNevin outlined, There were concerns raised that that there was risk for that threatened the collection of evidence. While I acknowledged this might be a risk, I believe we needed to look at the evidence and the risk and agreed it needed to be investigated. We had some discussions at two previous management meetings. I agreed that it could be a risk and it needed to be investigated."

The divergence of views and approaches is well illustrated in the evidence provided by two experienced scientists. Firstly, Valerie Caldwell from the Evidence Recovery Team outlined that, "In my view, Allan has acted appropriately in addressing the concerns raised in relation to the issues that commenced project #181. He copped flack from us in relation changing the testing but he also copped the flack from the other team in relation to the issue. He was in a very difficult position. I find that the major thing is that sometimes we rush to solutions when the problem isn't really understood which leads to having fix issues that haven't been considered. Also we are at the forefront of our field which leads issues where we might having teething problems." Whereas, Thomas Nurthen from the Reporting Team outlined, "As a reporting group, we identified there was an issue and as a group we met in May 2016. We knew of problems prior to this. A solution wasn't implemented until August. In my view we had enough information to act on it in May."

Reporting scientist, Jacqui Wilson, who was credited by Ms Reeves as the initial identifier of the problem stated that "I have raised the concerns with the team managers and then left them with them to manage. I not aware of evidence being missed but more that there was potential for evidence to be missed. I am aware that the project #181 was established to examine the issue. I understand that there needs to be gathering of information or more examples to move forward with that. I don't believe that there have been any deliberate roadblocks to addressing the issue."

Following consideration of the evidence, it is the view of the investigator that any perceived lack of progress on the Project #181, which was the responsibility of Mr McNevin, was not due to him being obstructive but was more concerned with the gathering of the evidence and analysing of the risk prior to reaching a solution.







There was no evidence presented to support the assertions of Ms Reeves that Mr McNevin deliberately failed to progress Project #181 nor that he caused a serious roadblock to process improvement that had the potential to put the organisation at risk.

Please note no submission or assertion was made during any interview that raised any concerns about the effectiveness of the 'workaround' to address the scientific concerns raised that resulted in Project #181.

# **Findings**

The allegation that Allan McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide and caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so is **not substantiated**.

# Other matters

## Amanda Reeves' response to Allan McNevin's email apology on the 9th of June 2016

Following the end of the management meeting, Ms Whelan, Mr Howes and Kirsten Scott (acting in Paula Brisotto's absence), met with Mr McNevin. Mr McNevin readily admitted that he had acted inappropriately and that he would apologise to Ms Reeves. Shortly after the meeting, Mr McNevin emailed Ms Reeves and offered to apologise in person for "spitting the dummy" in the management team meeting. Further, that he should not have let his frustration out like he did.

Ms Reeves responded by acknowledging the apology but declining to meet. Further, Ms Reeves responded by saying, "I can just tolerate you discounting my opinions and treating me with that vague sense of amused disdain, because mostly I don't care what you think of me, but I will not ever accept being physically or emotionally intimidated. You frightened me in that moment. I hope you feel like a big man."

Following receipt of this email, Mr McNevin forwarded it to his supervisors, Mr Howes, Ms Scott and Ms Whelan. As a result of this Ms Whelan sought a meeting with Ms Reeves with a view to discussing the incident and also Ms Reeves' email.

Prior to the meeting with Ms Reeves, Ms Whelan met with Mr McNevin. In that meeting Mr McNevin admitted his behaviour at the meeting was inappropriate but outlined that it was due to Ms Reeves repeatedly making the same point through the meeting. Mr McNevin also was concerned about Ms Reeves' email because he believed she intended to use it against him in the future. Further Mr McNevin sought a retraction of Ms Reeves' email and written apology as he believes there was no physical intimidation.

At interview, Ms Reeves outlined "I then received an email from Allan at 10.33am in which, rather flippantly in my opinion, Allan apologised for 'spitting the dummy at me'. I responded at 11.00am acknowledging his apology and letting him know, as the recipient of his behaviour, how it made me feel. I wouldn't meet with Allan because he had just physically and emotionally intimidated me and I didn't feel safe. When I left the meeting, I went and sat in a room and fell apart."

In a meeting with relieving Managing Scientist, Deb Whelan, Ms Reeves outlined, "Instead of being asked how I was and checking on my well-being, or being asked to contextualise the matter, I was reprimanded for my email response to Allan's apology email. Apparently Deb took exception to my words as they were too strong. I said that as the human being on the receiving end, this was how I felt."



At interview, Deb Whelan outlined, "Kirsten Scott sent Justin and I an email which outlined that Allan had apologised and offered to meet. Amanda's response was also included. Allan had forwarded both of the emails to Kirsten who forwarded it to us. What I noticed about the emails was that Amanda's last two sentences in her email were quite inflammatory and that Allan's apology in the email appeared quite flippant."

Mr McNevin outlined the following at interview, "While Amanda was gone, I was thinking about apologising and how I could say it. I knew she would be upset and I know how she had been upset in the past. The meeting concluded. Before I could say anything to Amanda she got up and left."

Further, "Following the meeting, when I returned to my desk I wrote an apology to Amanda. It wasn't received very favourably by Amanda. I thought about the email from Amanda and I felt she was accusing me of physical intimidation which was unwarranted it. I felt that she was being aggressive with the issue now by accusing me of physical intimidation. I was concerned that sort of allegation can cost me my career. I know I did the wrong thing but I didn't physically intimidate her."

Mr McNevin also reflected on this email in the following way, "my first apology was quite informal. I used the words 'dummy spit' – I have an informal way of writing emails but I understand the need for a more formal apology."

Ms Scott outlined, "From my point of view, Allan acknowledged he had made a mistake and acknowledged this. Apart from losing his cool, Allan couldn't have acted more appropriate to correct the matter. Amanda didn't appear to be ready to resolve the matter at that stage."

# **Assessment**

In the opinion of the investigator, both emails, Mr McNevin's apology after the incident and Ms Reeves' response were unfortunately sent when emotions were still raised following the incident. Mr McNevin intended the informal style of the email to de-escalate the situation but it had the opposite effect as Ms Reeves interpreted the apology as flippant. Ms Reeves' response outlined that she "will never accept being physically or emotionally intimidated. You frightened me in that moment. I hope you feel like a big man." Further, Ms Reeves declined Mr McNevin's offer to meet. Ms Reeves further outlined that "as the human being receiving end, this is how I felt."

It is the view of the investigator that while Ms Reeves may well have felt physically and emotionally intimidated by Mr McNevin there is a lack of evidence that Mr McNevin's conduct was physically intimidating or threatening (see Allegation 1). It is reasonable in her response to Mr McNevin to express how she felt and decline to meet with him. However, the last sentence, "I hope you feel like a big man" is not an expression of how Mr McNevin's conduct made Ms Reeves feel but a statement of belittlement towards Mr McNevin.

The email had the effect of making resolution of the matter very difficult from that point. Mr McNevin was adamant that his conduct was not physically intimidating and Ms Reeves refused to accept any apology from Mr McNevin that did not include acknowledgement that his conduct was physically intimidating.

The email response from Ms Reeves, while ill-considered and unhelpful in resolution of the conflict was sent in the heat of the moment so it could be a mitigation that Ms Reeves was in all probability highly emotional as a result of the incident. However, the statement in the email, "I hope you feel like a big man" is inappropriate and unprofessional.

In relation to Ms Reeves' general conduct in the workplace, there was sufficient testimony provided that Ms Reeves' communication style can be forceful and direct. Further, Ms Reeves' interactions with her colleagues was described as confrontational and challenging. There were elements in this in Ms Reeves' questioning of Mr McNevin which led to the incident in the management team meeting on 9 June 2016. HSQ



management may wish to consider whether Ms Reeves may benefit from some coaching in workplace communication.

#### Management action post incident

At interview Ms Reeves outlined that she believed management's handling of the matter was deficient and needed to be investigated.

The major issue for Ms Reeves is that she believes management inappropriately down played Mr McNevin's behaviour in the management meeting and escalated her email response to Mr Mc Nevin's original apology.

From a process point of view, management, in particular Ms Whelan who was acting as the Managing Scientist met with both parties separately on a number of occasions and facilitated the meeting on 8 August 2016 taking place. Ms Whelan was not present at the meeting on 8 August. Ms Brisotto returned from long-term maternity leave on 12 July 2016 and was therefore not present at the management meeting on 9 June 2016. Ms Scott relieved for Ms Brisotto. Upon her return, Ms Brisotto met with both Ms Reeves and Mr McNevin. Ms Brisotto then relieved for a short period as the Managing Scientist while Ms Whelan was on leave. During this time, following a discussion with Jade Franklin from Human Resources, Ms Brisotto attempted to organise resolution of the matter by a 'facilitated discussion' chaired by Mr Franklin. Following consideration, Ms Reeves declined to participate.

#### **Assessment**

Ms Whelan, Mr Howes, Ms Scott and Ms Brisotto were all interviewed. Ms Whelan was responsible for leading the management of the issue. Ms Whelan was relieving as Managing Scientist in the absence of the incumbent, Cathie Allen. Ms Whelan was unfamiliar with the team dynamics and personalities.

It is the view of the investigator that overall, the management team has genuinely attempted to resolve the matters in good faith. There have been numerous meetings with Ms Reeves and Mr McNevin. Issues raised by both parties have attempted to be addressed by the management team.

Upon reflection, Ms Whelan admitted that she should have dealt with some aspects differently. It is the view of the investigator that two aspects of Ms Whelan's management of the matter could have been approached differently. The first was that no manager checked in on the welfare of Ms Reeves after the incident. Ms Reeves and others were genuinely shocked and upset by the incident and therefore a manager should have checked in with her. The fact that Ms Reeves and others were shocked also supports how out of character this outburst was for Mr McNevin.

The second aspect of Ms Whelan's management of the matter that in hindsight could have been better handled was Ms Whelan's first meeting with Ms Reeves where the first issue raised by Ms Whelan was Ms Reeves' email to Mr McNevin. While Ms Reeves' email should have been raised during the meeting, as it was the first meeting about the issue, Ms Reeves' well-being should have been checked on. Further, in hindsight, it would have been more prudent to deal with the incident in the first instance. However, Ms Reeves' comments in the email, "I hope you feel like a big man" were derogatory towards Mr McNevin and made resolution difficult from that point on. This was not consistent with Ms Reeves' view that she was entitled to express how the incident made her feel.

Ms Whelan demonstrated an acute awareness of the flaws in her early approaches and is contrite upon reflection. Ms Whelan indicated that she had never confronted such a situation in all her time at Queensland Health. Further, Ms Whelan also stated that at the time, she was dealing with serious family issues and was quite preoccupied. The



investigator believes that Ms Whelan's early actions were as a result of misjudgement rather than of any act of negligence or malevolence towards Ms Reeves.

Apart from those early blemishes, the management of the matter has been reasonable and sound considering the difficult circumstances and approach to resolution by the parties. Senior management continued to meet with both Ms Reeves and Mr McNevin to attempt to reach resolution. This included proposals of mediation and a facilitated discussion, both of which were declined by Ms Reeves. This culminated in the meeting of 8 August between Ms Reeves and Mr McNevin, both with support people. While the meeting of 8 August did not go well, this was due to the entrenched positions of the parties which escalated the animosity rather than management's mishandling of this issue. The investigator is of the view that management of the matter was reasonable with exhaustive attempts to reach a resolution.

# The Forensic DNA Analysis Management Team

During interviews with management and staff, interviewees were asked by the investigator to reflect on the functioning of the management team. Those interviewees who had attended management team meetings also commented about the conduct and effectiveness of those meetings.

The major concern consistently expressed was that the management team is split into two groups. The split is between the analytics/evidence recovery area and the reporting team. This was also noticeable to employees who do not attend the management team meeting. Some of those who attend management team meetings expressed concerns that the two groups become quite positional in their approach to issues. This risks issues not being addressed on their merits but rather a position being taken based on team loyalty.

Another concern raised is that there is a lack of communication from the management team to employees. A common comment is that information is often on a 'need to know basis' which doesn't filter to employees.

There was also a view expressed that members of the management team do not receive support and training their role as managers. Former team leader and experienced scientist, Thomas Nurthen outlined that when he commenced work in 2004 the workplace was 'very dysfunctional'. Mr Nurthen went on to say that a program of team building was implemented which was successful for a period of time.

Submissions were made that managers were sometimes 'thrust' into a management position without ongoing support. Further, that there was not a program of ongoing support or a management development program. Mr Nurthen, no longer a member of the management team, also made the suggestion that the management team would benefit from having a greater appreciation of what other teams do.

#### **Assessment**

The evidence presented to the investigator indicates that the management team are split into two groups and that management team meetings can be divided and quite confrontational. Further, that the members are quite positional in dealing with issues. There was no evidence presented however that any member of the management is not dedicated to ensuring the DNA Analysis Unit providing an excellent service. This gives the management team a solid basis to work from in that this is a common interest for all management team members.

Upon consideration of the evidence presented, the investigator believes that it may be worth considering changing how the management team meetings operate and approach issues. HSQ may wish to consider introducing an 'interest-based' approach for the management team to address issues raised at management team meetings. This will assist the management team to deal with matters on a consensus basis while considering specific interests of



management team members. The investigator is of the opinion that the divergence of the team in project # 181 may well have been avoided if all of the parties' interests and concerns were understood and appreciated by all parties. Further, to assist in this process, an independent chair, experienced in the 'interest-based' process may be considered.

In relation to management support, HSQ way wish to consider whether a formal leadership/management support program should be introduced. The program may consist of a 360 degrees feedback, leadership values and coaching/mentorship initiatives.







# **Summary of Findings**

#### **Background**

On 24 October 2016, Livingstones was appointed by the Chief Executive Officer, Heath Support Queensland in accordance with the *Hospital and Health Boards Act 2011* to investigate and report on matters related to the management team of Forensic DNA Analysis at Forensic and Scientific Services as outlined in the Terms of Reference. This arises from an incident on 9 June 2016 between Allan McNevin and Amanda Reeves at the management team meeting. Both Mr McNevin and Ms Reeves are supervising scientists of their respective teams and members of the Forensic DNA Analysis management team.

## **Allegations**

# **Allegation One**

Amanda Reeves alleges that Allan McNevin's response to feedback provided by her in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours.

# **Finding**

The allegation that Mr McNevin's response to feedback provided by Ms Reeves in a meeting held on 9 June 2016 was intimidating and an unacceptable escalation of his existing behaviours is **not substantiated**. However, there is sufficient evidence, including Mr McNevin's admission that he shouted at Ms Reeves which is not consistent with the *Code of Conduct for the Queensland Public Service section 1.5 'Demonstrate a high standard of workplace behaviour and personal conduct.* 

# **Allegation Two**

Amanda Reeves alleges that Allan McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships.

# **Finding**

The allegation that Mr McNevin's determination to take and immovably maintain a position and associated unwillingness to be open to feedback or adjust his attitudes and behaviours to improve the working relationships is **not substantiated**.

#### **Allegation Three**

Amanda Reeves alleges that an email from Justin Howes, Team Leader to all reporting staff on the 5 of August 2016 regarding standardised statement wording was specifically directed at Mr McNevin.

# **Finding**

The allegation that an email from Justin Howes, Team Leader to all reporting staff on the 5 of August 2016 regarding standardised statement wording was specifically directed at Mr McNevin is **not substantiated.** 

# **Allegation Four**

Amanda Reeves alleges Allan McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide and caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so.



#### **Finding**

The allegation that Allan McNevin failed to progress the project regarding Project #181 Spermatozoa seen on Differential Lysis extraction slide vs Evidence Recovery suspension slide and caused a serious roadblock to process improvement and potentially put the organisation at risk by doing so is **not substantiated**.

#### Other matters

## Amanda Reeves' response to Allan McNevin's email apology on the 9th of June 2016

The email response from Ms Reeves, while ill-considered and unhelpful in resolution of the conflict was sent in the heat of the moment so it could be a mitigation that Ms Reeves was in all probability highly emotional as a result of the incident. However, the statement in the email, "I hope you feel like a big man" is inappropriate and unprofessional.

In relation to Ms Reeves' general conduct in the workplace, there was sufficient testimony provided that Ms Reeves' communication style can be forceful and direct. Further, Ms Reeves' interactions with her colleagues was described as confrontational and challenging. There were elements in this in Ms Reeves' questioning of Mr McNevin which led to the incident in the management team meeting on 9 June 2016. HSQ management may wish to consider whether Ms Reeves may benefit from some coaching in workplace communication.

# Management action post incident

Despite some early blemishes, the management of the matter has been reasonable and sound considering the difficult circumstances and approach to resolution by the parties. Senior management continued to meet with both Ms Reeves and Mr McNevin to attempt to reach resolution. This included proposals of mediation and a facilitated discussion, both of which were declined by Ms Reeves. This culminated in the meeting of 8 August between Ms Reeves and Mr McNevin, both with support people. While the meeting of 8 August did not go well, this was due to the entrenched positions of the parties which escalated the animosity rather than management's mishandling of this issue. The investigator is of the view that management of the matter was reasonable with exhaustive attempts to reach a resolution.

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# **Signatures**

Investigators Name: Mark Brady

Signature:

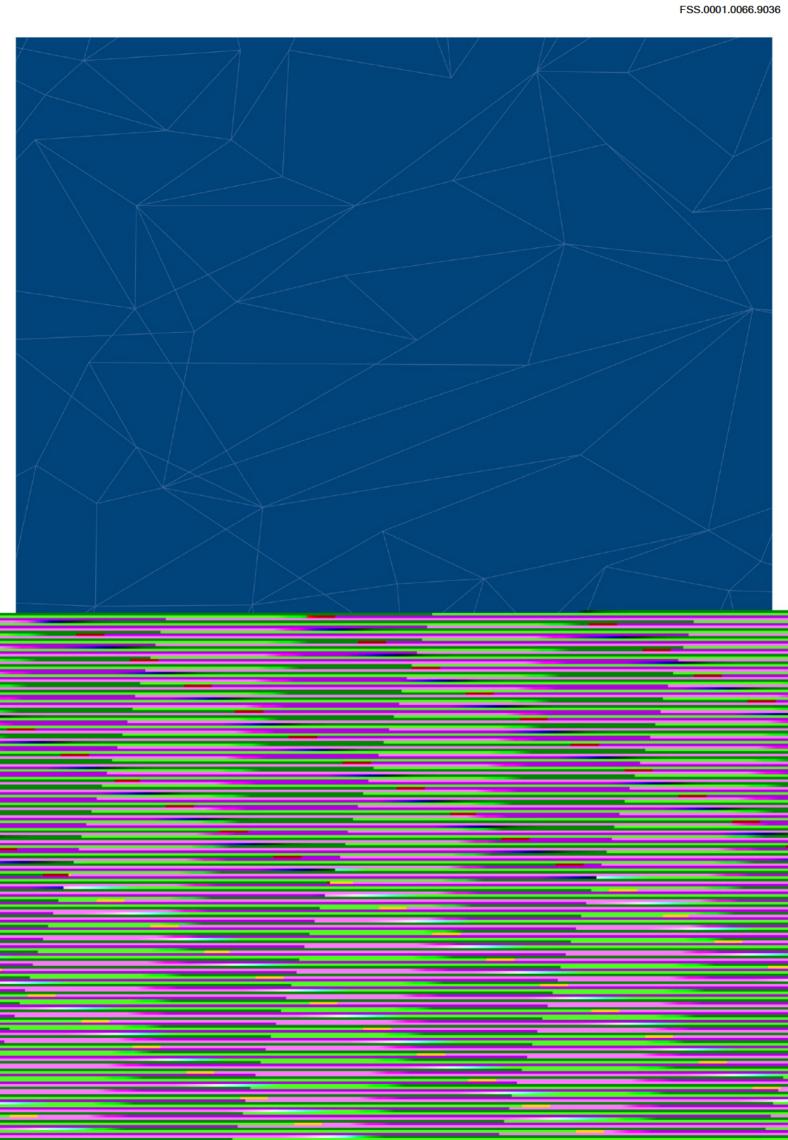


Position/Title: Principal Consultant

Livingstones

Date: 17 February 2017





# **Cathie Allen**

From: Paul Csoban

Sent: Monday, 27 March 2017 8:00 AM

To: Louise Syme

Cc: Sandy Sinclair; Cathie Allen
Subject: RE: FSS - Legal and Priveleged

## Hi Louise

Thank you. We have a car park booked but unfortunately today of all days I left my ID at home so you will have to send someone to escort us.

See you soon

Paul

From: Louise Syme [mailto:

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## Hi Paul

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I understand that a car park has also been booked for you. Please let me know if you haven't already received the details for parking in our building.

# Kind regards

Louise

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Senior Principal Lawyer

Advocacy - Galligan Chambers



From: Paul Csoban [mailto:

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To: Louise Syme <
Cc: Sandy Sinclair < Cathie Allen <

Subject: RE: FSS - Legal and Priveleged

# Hi Louise

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It may well be worthwhile for you to bring in someone from employment law as the 2 are intermixed. However again the reason for approaching you is the issue of legal prosecution. It is rather complex to explain in writing. I will be bringing along my managing scientist in charge of our police stream.

Kind regards

Paul



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**Executive Director** 

Forensic and Scientific Services Health Support Queensland, Department of Health

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From: Louise Syme [mailto:

Sent: Friday, 10 March 2017 11:56 AM

To: Paul Csoban

Subject: RE: FSS - Legal and Priveleged

Thanks Paul

All the best for this afternoon.

Kind regards Louise

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### Hi Louise,

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Thank you in the interim

Regards Paul

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Thanks Paul

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My concern is largely around her suitability to give evidence on these types of cases given her overt and documented questioning of the outcomes of the tests. She has clearly stated that she will not accept the outcomes of the scientific review if it contradicts her views.

In any event we have a *Without Prejudice* meeting with Amanda and her legal representatives this afternoon (Clayton Utz will also be attending on our behalf) and perhaps I can contact you after that to work out the next steps. Would it be feasible after that to come and meet with you (if required) to fully explain my concerns in this matter and answer any questions that may still arise?

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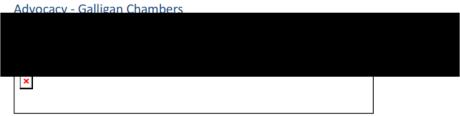
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Would you be content for me to forward your email to our colleagues in the employment law team for them to provide the advice you seek regarding the direction to Ms Reeves that she is not to give expert evidence at present and any other steps you may take in relation to any overtures she may make about giving evidence or making a PID?

Kind regards Louise

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From: Paul Csoban <

Sent: Wednesday, 8 March 2017 9:47 AM

To: Louise Syme

Subject: FW: FSS - Legal and Priveleged

From: Paul Csoban

Sent: Thursday, 2 March 2017 1:12 PM

Subject: FSS - Legal and Priveleged

Importance: High

Hi Louise,
Thank you for taking my call regarding advice on a serious matter.
I have attached two documents which outline the issues in the matter of Amanda Reeves.
Brief to DG as a preliminary in case of PID Letter from CEO outlining issues and actions taken.
It should be noted that further correspondence has occurred and it appears that Amanda is engaging further counsel with the statement (from current lawyer) that she will not accept the findings of the independent scientific review from ESR (Institute of Environmental Science and Research Limited) whatever they may be, as she hadn't been interviewed on the matter. This is again in contradiction to her previous statements that she is fully accepting of the current scientific process
Further, Clayton Utz have been engaged by HSQ and are currently handling the HR side of things.
Please call me of let me know if you have any further questions, as I appreciate the matter is quite complex.
Regards
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From: Cathie Allen

Sent: Fri, 10 Mar 2017 11:01:02 +1100

To: Shae McCartney

Cc: Paul Csoban; Jade Franklin

Subject: FW: Additional information

Attachments: 17189V9\_20070724.doc, 17189V10\_20100920.doc

# Hi Everyone

It has been stated that the procedure used to process samples for sperm was changed in 2008 - so there is a question about sample processing between 2008 and August 2016 (when the risk mitigation step was introduced).

I've compared the SOPs used and the new process where water was added to a swab in a tube and then the water used to make a smear (instead of wetting the swab and rolling the swab on the slide) was introduced in September 2010. I've attached the SOPs and highlighted the change in yellow usually on page 1 of the SOP.

# Cheers Cathie



# Cathie Allen

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health

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From: Cathie Allen

Sent: Thursday, 9 March 2017 1:57 PM

To: Shae McCartney ( Cc: Paul Csoban; Jade Franklin; Subject: Additional information

Hi Shae

The SOP used to detect sperm in sexual assault cases contains an amendment history (section 11), this shows that the process hasn't vastly changed in the period between 2008 and 2016. The PowerPoint attachment (Details for Sperm SOP) shows that Amanda Reeves has had the opportunity to have input into the procedure over a number of years. She was a reviewer of the SOP, then a notifee of all updates to the SOP (as most other staff are as well).

As discussed by phone with Joanne – I've attached a document which shows when we would use the different SOPSs. Plus also a Team Chart – how the teams are set up on the page (L to R) is how a sample moves through our processes (without going through the Quality & Operational Team specifically).

Adverse event and their guidelines – I've attached the SOP for investigating Adverse Events, however this issue has never been deemed an adverse event as we have no evidence to suggest that there is a gross or systemic issue with the procedure. Whilst a couple of cases have been put forward as showing no sperm detected at Evidence Recovery stage, but a DNA profile was obtained and review of the slides from the Analytical process showed sperm – this could be due to human error as well as other factors that may have affected only that sample. Other examples of an adverse event in Forensic DNA Analysis are: in 2008, an instrument did not operate at an optimal level and sample to sample contamination was discovered. This affected samples that had been processed for a period of time and required advice to both QPS and DPP from myself and the Senior Director. Another example was a minor miscode in STRmix (software used to generate likelihood ratios for DNA profiles). A new version was released, however 24 Statements had to be re-issued as the stats had changed with the new version and again, required advice to both QPS and DPP from myself and the Senior Director.

Jacqui Wilson raised the issue to her line manager, Amanda Reeves, a case that she was reporting on (email from 4<sup>th</sup> of March 2016) had minimal sperm detected at Evidence Recovery phase of the process but larger numbers of sperm at Analytical phase. Jacqui has been interviewed by the External Investigator and she has verbally advised me that she has no issue with the processing that is undertaken in the lab regarding sexual assaults (or any other sample).

Staff had a small round table discussion about the processing of Sexual Assault Investigation Kits (SAIKs) – they were: Anna Lemalu (now works at ESR NZ and we've just been thanked for the excellent training that we gave her as she's fitted seamlessly into their workplace), Adrian Pippia (interviewed by Ext Inv), Thomas Nurthen (interviewed by Ext Inv), Jacqui Wilson (interviewed by Ext Inv), Matthew Hunt (currently acting in Amanda's HP5 position and has been supportive of all our efforts with Project #181 etc), Josie Entwistle and Penelope Taylor (neither have been interviewed as they are in a different reporting team). The list of suggestions they put forward was:

- looking at the slide making process in the Evidence Recovery phase of the process
- data mining (this is being done from the mitigation step so all samples have been submitted for profiling regardless of sperm detection since August 2016 data suggests that there is no gross / systemic failure of the process)
- check on the amount of liquid added to swab at the Evidence Recovery phase of the process (Justin Howes has looked at journal articles and has found that of the articles that list the amount we add less volume as we are trying to converse the amount of evidence in the sample so we're trying not to dilute it too much so that we detect sperm)
- suggested making control swabs and following them through the process (this is essentially what Project #181 is doing)

Amanda has supplied 'evidence' to her lawyer of another staff member questioning the process - Emma Caunt. Emma is currently doing Project #181 in conjunction with Allan McNevin. The

evidence supplied was from a Lynx instant message. Kylie Rika is a Emma's line manager, and Emma and Amanda are friends outside of work. Kylie has not raised any issues from her team regarding this process.

Amanda has acted in the role of Team Leader – Forensic Reporting and Intelligence Team for a two week period in June 2016 (EMF attached) – during this time, she could have used the authority of that position to implement changes or request work to be done to ascertain if a gross or systemic failure of the process was occurring. In a previous higher duties period, Amanda used the NCIDD results to conduct a familial search – authority for which was only held by Team Leaders and above. A familial search of NCIDD can only be authorised by the QPS or Team Leader or above for a particular matter – as the Qld legislation is silent on familial searching. This issue was investigated at the time, and as Amanda supplied the SOP that stated Team Leaders had the authority, no further action was taken.

Amanda is also the Author responsible for the Evidence Recovery processes for reporting scientists training module – I've attached that Training Module #28079. This means that she should have a very good understanding of all the processes in the Evidence Recovery portion, so during formulation of this training module, she may have reviewed documents including the sperm process. This document was first introduced in 2013.

I've attached the Australian and New Zealand Forensic Science Society (ANZFSS) code of conduct. I'm fairly sure that Amanda is a current member. The code discusses – acting 'truthfully and objectively' – given that ESR have said that we have a sound, scientific procedure, if Amanda were to not accept this, then perhaps she's not being objective (I understand that she would need to have access to the report or a summary thereof).

The court expects that an expert witness will provide testimony that is fact based, and if the expert offers 'opinion based' testimony then they should clearly state that it is opinion based. The facts as presented in a Statement of Witness are that a biological matter was identified (or a sample process with the assumption of biological material – eg cells due to someone touching an object) and a DNA profile obtained, with statistics on the likelihood of it being from a particular person. How that biological matter was deposited is an opinion.

Amanda Reeves supplied controlled documents (SOPs) and minutes of Management Team Meetings to her counsel. Amanda did not ask permission to supply these documents to anyone.

Please let me know if there's anything else that I can supply or provide detail on.

# Cheers Cathie



# Cathie Allen

Managing Scientist - Police Services Stream

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# **Cathie Allen**

From: Cathie Allen

Sent: Tuesday, 28 March 2017 5:18 PM

To: Paul Csoban

**Subject:** RE: Confirmation of instructions.

# Hi Paul

I've tracked my changes below.

# Cheers Cathie

# 7

# Cathie Allen

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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From: Paul Csoban

Sent: Tuesday, 28 March 2017 2:37 PM

To: Cathie Allen

Subject: FW: Confirmation of instructions.

Hi Cathie

Can you please reviews and send back any amendments to me please.

I will do the same

Thanks Paul

From: Louise Syme [mailto:

**Sent:** Tuesday, 28 March 2017 1:44 PM

To: Paul Csoban

**Subject:** Confirmation of instructions.

Dear Paul,

Thank you for your time yesterday. I have provided below a summary of the information you provided yesterday and framed a number of questions to be answered in our advice. Could you please advise whether the summary and draft questions accurately reflect your concerns?

# Background

Scientists employed within the Forensic Reporting and Intelligence Team (Reporting Team) of Forensic and Scientific Services (FSS) are responsible for preparing reports to the Queensland Police Service, providing scientific [witness] statements and appearing to give expert evidence as required.

The FFS FSS has had a standard operating procedure (SOP) for the analysis of sexual assault kits. Prior to 2008 that SOP involved the following steps (the old process):

- 1. The swab is removed from its swab casing vial, moistened with distilled water and rolled abbed on a microscope slide. If sperm was identified on that slide, the swab was sent for DNA analysis.
- 2. If sperm was not identified on that slide, water would be added to the swab and the swab would be rolled across paper. Presumptive testing would then be conducted on that paper. If sperm seminal fluid was detected identified on the paper, the swab would be sent for DNA analysis.
- 3. If no sperm seminal fluid was identified in the presumptive test, the swab would then be used for cell testing.

In June 2010 the SOP was amended to involve the following steps (the new process):

- Dilution of the swab in a the vial with nanopure water and mixed. in which it was provided. The swab was
  then removed from the vial and rolled across A drop of the water is removed and placed onto a microscope
  slide (the slide test). If sperm is identified on that slide, the swab is sent for DNA analysis.
- 2. If sperm is not identified on that slide, the swab is be returned to the vial and the vial would be "vortexed" in the hope to extract sperm that had penetrated the swab. A sample of the fluid after vortexing is used for presumptive testing. If sperm seminal fluid is identified on that test, the swab is sent for DNA analysis.
- 3. If no sperm or seminal fluid is identified in either the slide test or the presumptive test, the swab is then be used for cell testing.
- 4. Swabs sent for DNA analysis whereby both the swab and the remaining fluid are processed to separate the sperm from epithelial cells. During this processing, sperm are spun to the bottom of the tube before another slide is prepared, then undergo a procedure to remove the water used for dilution-thereby returning the any sperm sample to its concentrated state.

The new process was introduced with a view to preserving as much sample as possible for DNA profiling whilst still undertaking a slide test and presumptive tests. larger sample for testing and analysis. As the new process retains the fluid and dilutes the sperm sample, where only a low number of sperm have been collected presumably only a small number of sperm will be used in each stage of testing- allowing for a greater number of sperm to be available for DNA analysis if appropriate.

The SOP remained largely unchanged until August 2016 when further risk mitigation processes implemented to ensure that all samples were processed were written into the procedure. Whilst the SOP was amended in 2010, the manual detailing the SOP was not amended until August 2016. I'm not sure what you're trying to say. The new process was implemented and documented in the SOP in 2010. A risk mitigation step wasn't implemented until August 2016. The SOP has been updated on the following occasions: 20/09/2010, 22/02/2011, 15/11/2013, 29/07/2015 and 09/03/2017.

A member of the Reporting Team (the scientist) raised concerns regarding the new process being "bad science" around March June 2016. In response to those concerns the FFS implemented risk minimisation processes for the analysis of sexual assault kits in August 2016, after some preliminary investigation into the process. In particular, the FFS has been sending swabs for DNA analysis irrespective of whether sperm was identified on the slide test or presumptive test. As a result, approximately 650 swabs have been analysed with approximately 2% (approximately 13) of those swabs being found to contain sperm. At present the FFS cannot confirm whether positive DNA analysis has been possible on those swabs. These results are still being reviewed and a report will be compiled in the next 2 weeks.

The scientist made complaints regarding the personal conduct of a colleague in August November 2016. Since that time the scientist has made claims for personal injury and been involved in meetings with the FSS regarding her concerns. Crown Law is not instructed to act in relation to any of those matters.

In the course of her communications with the FSS regarding her complaints and injury claims, the scientist has also raised concerns that the FSS has been relying on "bad science" for the period between 01/01/2008 June 2010 and August 2016 present. Those concerns include, but may not be limited to, concerns that:

- The new process has resulted in sperm samples not being effectively detected by the slide test or
  presumptive test and therefore samples are not being sent for DNA analysis and evidence is not detected.
  being ever diluted with the effect that some samples have not been sent for DNA analysis because the
  diluted sample did not result in the identification of sperm on either the slide test or presumptive test.
- 2. As the new process is not as effective, the samples most at risk of being missed, as the samples were low numbers of sperm are. The new process won't detected that there's sperm or seminal fluid and therefore won't be sent for DNA analysis. As the sperm sample is more diluted using the new process, there is a lower probability of sperm being identified on the slide test or presumptive test and therefore an increased probability that swabs containing low sperm numbers are not being sent for DNA analysis.
- There may be 60 cases which require re-examination because of an ineffective process over dilution. (However, the basis for this figure has not been explained or investigated.)

In response to the concerns raised by the scientist, FSS increased its risk minimisation processes in August 2016. The FSS has also sought a review by the Institute of Environmental Science and Research Limited (ESR) and undertaken complementary internal work testing the science. The FSS remains of the view that the new process represents the best process currently available for the analysis of sexual assault kits and a practice consistent with those used in benchmark organisations.

After a period away from the workplace, the scientist has returned to work and is undertaking a project in another forensic field. She has indicated that she wishes to return to her substantive role, including undertaking work as an expert witness for the FSS. The scientist has previously indicated that she would be satisfied that the new process is appropriate if the ESR report supports that position, however that position has now changed, although the scientist hasn't been provided with the outcome of the ESR review. However, the FSS is concerned that the scientist has been inconsistent in her position over the new process. Accordingly, the FSS has ongoing concerns about the evidence the scientist may give in sexual assault prosecutions and the impact of that evidence on the scientist's reputation and effectiveness as an expert witness, the outcome of prosecutions (future and past) and the reputation of the FSS.

We are asked to advise on the following:

- 1. If the scientist gave evidence that the new process was "bad science", what would be the effect of that evidence on:
  - a. the relevant prosecution;
  - b. the outcome of past prosecutions;
  - c. the reputation and effectiveness of the scientist as an expert witness; and
  - d. the reputation of the FSS and effectiveness of other FSS scientists as expert witnesses.

I would be happy to discuss any of this information as required.

Louise Syme Senior Principal Lawyer



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# **Cathie Allen**

From: Paul Csoban

Sent: Friday, 7 April 2017 5:19 PM

To: Gary Uhlmann
Cc: Jade Franklin

**Subject:** Amanda Reeves meeting and Crown Law document

Attachments: Crown Law Advice\_Amanda Reeves.pdf

**Importance:** High

Hi Gary,

As per our phone conversation I attach advice from Crown Law regarding circumstances around Amanda's claims regarding DNA process.

Jade, Shae and I met with Amanda and her lawyer for several hours this afternoon to discuss aspects around her acceptance and willingness to abide by the outcomes of both the Livingstone's Review and ESR Scientific Review. Amanda was very circumspect and evasive with her answers and would not give a firm and definitive commitment to returning to work in a harmonious and professional capacity and accepting all the grievance issues outlined previously as settled.

Shae (Clayton Utz lawyer) had a *without prejudice* discussion with her lawyer after the meeting and he committed to reverting to us on Monday afternoon with any potential alternatives to a complete return to her substantive role in DNA.

In summary, I am not convinced that Amanda has the desire and willingness to return in her substantive role and operate in a professional and committed manner and to observe all Code of Conduct requirements. Her answers, demeanour and behaviour during this and previous discussions demonstrated quite the reverse in my opinion. I believe she could raise similar issues in the future and could potential cause great harm to the DNA unit in which she works and possible to the reputation of FSS.

I recommend we await the response from Amanda's lawyer on Monday and then convene a meeting with Shae, Di, Jade me and you to determine our best course of action

Kind regards Paul

# **Paul Csoban**

Executive Director | Forensic and Scientific Services | Health Support Queensland Department of Health | Queensland Government 39 Kessels Road Coopers Plains QLD 4108





# **Cathie Allen**

From: Cathie Allen

**Sent:** Thursday, 4 May 2017 12:17 PM

To: Paula Brisotto

Subject: RE: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries

and Reviews

### Excellent

# Cheers Cathie



# **Cathie Allen**

A/Executive Director

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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From: Paula Brisotto

Sent: Thursday, 4 May 2017 12:14 PM

To: Cathie Allen

Subject: FW: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

# How's this?

Quality review of processes regarding sexual assault examinations and detection of spermatozoa to address concerns raised internally. The review was performed by the Institute of Environmental Science and Research, New Zealand, who undertake similar testing to the Forensic DNA Analysis, PSS, and are well respected in the Forensic Science community and are considered to have expertise in this area.

# Paula Brisotto

A/Managing Scientist
Police Services Stream
Forensic & Scientific Services,
Health Support Queensland, Department of Health

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From: Paula Brisotto

Sent: Thursday, 4 May 2017 11:55 AM

To: Cathie Allen

Subject: FW: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

Hi Cathie,

FYI

Paula



# Paula Brisotto

A/Managing Scientist
Police Services Stream
Forensic & Scientific Services,
Health Support Queensland, Department of Health

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From: Paula Brisotto

Sent: Thursday, 4 May 2017 11:34 AM

To: Paul Csoban

Subject: RE: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

Hi Sandy,

A review performed in Forensic DNA Analysis, Police Services Stream is as follows:

- Brief context/background
   Quality review of processes regarding sexual assault examinations and detection of spermatozoa
- Current status of the review Completed
- Anticipated completion date of review N/A
- Anticipated completion date for implementation of recommendations

Recommendations are currently being considered, and will be incorporated into standard operating procedures as appropriate.

Regards, Paula



# Paula Brisotto

A/Managing Scientist
Police Services Stream
Forensic & Scientific Services,
Health Support Queensland, Department of Health

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From: Sandy Sinclair On Behalf Of Paul Csoban

Sent: Wednesday, 3 May 2017 4:39 PM

To: Adam Griffin; Charles Naylor; Claire Dolereit; Deborah Whelan; Helen Gregg; Lee Smythe; Paula Brisotto

Cc: Cathie Allen

Subject: FW: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

Hi Everyone

Are you aware of any reviews/inquiries in FSS in the last 12 months (excluding QAO) and if so can you please provide the following information:

Brief context/background
Current status of the review
Anticipated completion date of review
Anticipated completion date for implementation of recommendations

Otherwise please provide a Nil response by Tomorrow 4 May 12 Noon.

Thank you, Sandy

From: HSQ-CSS

**Sent:** Wednesday, 3 May 2017 2:12 PM **To:** Victoria Chalmers; Paul Csoban

Subject: FW: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

Hi Victoria and Paul

Do you have anything for this? CFMU review was earlier – just need for this fin year.

Thanks Cheryl From: HSQ-Governance

Sent: Wednesday, 3 May 2017 10:21 AM

To: HSQ-CSS; HSQ-FaBS; HSQ-GMPPE; HSQ-HSS; HSQ-PP; HSQ Customer Experience

Cc: HSQ-Corro; HSQ-OCE; Michael Speter

Subject: Fy input [Due 4/5]: IN170243 - Estimates brief input required - 32.02 Inquiries and Reviews

# **Good morning Teams**

The Estimates Team have requested for HSQ to provide input into the estimates brief on reviews and/or inquiries. The Governance Team is coordinating HSQ's response on behalf of the HSQ Corro Team.

Could you please advise of reviews/inquiries that you are aware of in the last 12 months (excluding QAO) and provide the following information:

- Brief context/background
- Current status of the review
- Anticipated completion date of review
- Anticipated completion date for implementation of recommendations

Otherwise please provide a Nil response.

If you could please respond to HSQ-Governance by COB Thursday 4 May, that would be greatly appreciated.

Happy to discuss further if required.

# Regards Laura



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# **Paul Csoban**

From: Patrick Steele

**Sent:** Tuesday, 18 July 2017 9:56 AM **To:** Paul Csoban; Karyn Bell

Subject: RE: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Thanks Paul – I believe I have a copy so I will go through that

# Cheers



## Patrick Steele

Director, HR Performance

People Performance and Excellence Health Support Queensland, **Department of Health** 

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From: Paul Csoban

Sent: Tuesday, 18 July 2017 9:53 AM

To: Patrick Steele; Karyn Bell

Subject: RE: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Pat,

# To clarify further:

The review was actually conducted on the DNA unit as AR claimed "systemic" problems with HR but specifically addressing her interactions and responses to the particular HR issue. I have a copy if you require Paul

# Hi Pat,

The HR Investigation Report was conducted by Livingstons in response to the 4 broad allegations made by AR around the various HR issues and the manner n which she was treated. All were found to be unsubstantiated. There was also a report by ESR on scientific basis surrounding her allegations of "poor science" and possible errors in results. This too confirmed FSS position.

Fully agree for you to draft another submission (although I do note that the time line for review have elapsed) and Karyn and I can review it for historic accuracy.

# Regards Paul

From: Patrick Steele

Sent: Tuesday, 18 July 2017 9:28 AM

To: Karyn Bell; Paul Csoban

Subject: FW: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Hi Paul/Karyn

I have confirmation that the timeline of events was all that was submitted by Jade.

I will review Ms Reeves' appeal submission and see if we need to provide further information but think it is likely.

I'm uncertain as to what is meant by the reference to "HR Investigation Report" – do either of you know?

## **Thanks**

## Pat



# **Patrick Steele**

Director, HR Performance

People Performance and Excellence Health Support Queensland, **Department of Health** 

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From: Kara Frederiksen

Sent: Tuesday, 18 July 2017 9:12 AM

To: Patrick Steele

Subject: RE: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Hi Pat,

The timeline of events is what was provided. And noting that most of Amanda's original submission as I understand it from Jade, was initial components of statements/info given to Livingstones (?)

I have also now attached:

- Amanda's original submission to WorkCover
- My correspondence to FSS re preparing the Employer response
- Another copy of the timeline provided as the Employer response
- And again the Review submission that Amanda has put in

# Happy to discuss

# Regards



# Kara Frederiksen

Principal Advisor Rehabilitation and Wellbeing

People Performance & Excellence Health Support Queensland, **Department of Health** 

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From: Patrick Steele

Sent: Monday, 17 July 2017 12:05 PM

To: Kara Frederiksen

Subject: RE: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Thanks Kara

Can you confirm/send the original HSQ submission to WCQ - or was it simply that timeline of events?

Cheers

Pat



# **Patrick Steele**

Director, HR Performance

People Performance and Excellence Health Support Queensland, **Department of Health** 

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From: Kara Frederiksen

**Sent:** Monday, 17 July 2017 10:21 AM **To:** Paul Csoban; Patrick Steele; Karyn Bell

Subject: CONFIDENTIAL - A REEVES - REGULATOR REIVEW OF WC DECISION

Hi Paul, Patrick and Karyn,

As previously mentioned, Amanda Reeves has applied to the Regulator for a review of WorkCover's decision to reject her workers compensation claim. A Review Officer has now been assigned and any further response you may wish to provide is due by **24 July 2017.** 

I have attached:

- The initial Employer response that was submitted (via Jade)
- The submission that Amanda has put forward to the Regulator for the review

In reviewing the decision, the Regulator has advised the issues for determination at review are:

- Did Ms Reeves sustain a personal injury of a psychological/psychiatric nature;
- Did the personal injury arise out of, or in the course of, Ms Reeves' employment;
- Was employment the major significant contributing factor to the development of the personal injury;
- Did the personal injury arise out of, or in the course of, management action; and
- Was the management action reasonable and taken in a reasonable way.

In terms of any further response you might like to provide, I suggest that if there is any further information available from the HR investigation that was conducted that may speak to management actions taken to address the issues, then this would be most relevant. The definition of injury precludes reasonable management action taken in a reasonable way.

Please note that under Procedural Fairness, the Regulator may share any further documentation provided with Amanda, as they have done in sharing Amanda's submission for review with HSQ.

If you would like a teleconference to review and discuss just let me know and I will arrange.

Regards

Kara



# Kara Frederiksen

Principal Advisor Rehabilitation and Wellbeing

People Performance & Excellence Health Support Queensland, **Department of Health** 

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# **Paul Csoban**

From: Paul Csoban

**Sent:** Friday, 21 July 2017 2:34 PM

To: Karyn Bell

**Subject:** FW: REEVES: Draft submission to Workers Comp Regulator

**Attachments:** Amanda Reeves - Workers Comp Regulator submissions 21072017.doc

# Hi Karyn

# My comments as additions

- 1. The initial application for Workers Compensation was made many months after the incident. From memory, I think one of the reasons for this was stated by AR that the consequent investigation by Livingstons exacerbated her injuries. However it should be noted that she apparently either requested or certainly was supportive of the investigation into the systemic problems in her unit. The outcomes of the review did not substantiate any of her 4 allegations.
- 2. After taking some time off, she has returned to the workplace and completed valuable work without any apparent problems or evidence of injury.
- 3. As a consequence of further allegations in the course of the investigations, a scientific review was conducted by ESR (again with her enthusiastic approval) which also did not substantiate her serious allegations about the quality of scientific work being performed in one particular area.

Happy for you to amend

Regards

Paul

From: Patrick Steele

**Sent:** Friday, 21 July 2017 1:27 PM

To: Paul Csoban; Karyn Bell; Kara Frederiksen

Cc: Karen Davies

Subject: REEVES: Draft submission to Workers Comp Regulator

Hi all

Please see attached draft submission.

I'm happy with whatever amendments you think need to be made.

Once completed, Kara can you please send on?

**Thanks** 

Pat



4 August 2017

Enquiries to: Mr Shaun Mulholland

Acting Manager,

Performance and Conduct

Services

Human Resources Branch Corporate Services Division

Telephone:

File Ref: C-ECTF-17/3581

Ms Amanda Reeves

Email:

Dear Ms Reeves

Thank you for your letter received 20 June 2017, regarding a workplace issue associated with concerns which you raised relating to a scientific process.

In your letter you indicated the matters raised by you may constitute a public interest disclosure (PID). The Ethical Standards Unit (ESU), Department of Health, has carefully considered all the available information and as a result, has assessed that the matter does not meet the definition of a PID as expressed in the *Public Interest Disclosure Act 2010*. The ESU has also assessed that the matter does not raise a suspicion of corrupt conduct as defined in the *Crime and Corruption Act 2001*.

I have carefully considered the concerns you have raised and recognise that you have an honest belief that certain administrative decisions and conduct have had an adverse effect on you in the workplace. Consequently I accept your letter, in principal, as a complaint.

I have delegated Ms Barbara Phillips, Deputy Director-General, Corporate Services Division, to be the decision maker in this matter. Ms Phillips will consider your complaint in detail and make the necessary enquiries to determine what, if any, action is required, and provide you with an outcome.

You are required to keep the details of your complaint confidential. However, you may discuss the matter with your support person, union representative or Employee Assistance provider. If you need to discuss this matter with any staff member you should make this request through Mr Shaun Mulholland, Acting Manager, Performance and Conduct Services, Human Resources Branch, Corporate Services Division.

Should you require any further assistance at this time, the Department of Health's contact is Mr Mulholland, on telephone or email

Yours sincerely

Michael Walsh Director-General Queensland Health

Page: write HR Chad OF + COA 22/03/2017 ATO - instrument its in - - Don't have an arrangement. - JF following up on this. EB8 - variable - Ru & Half Pay - operational convenience doesn't TOIL allow to facilitate this regrest, there adequately 18-4 planned for 4mks/year, workload isaes. - Sick leave - re: capacitation, shouldn't be pre-booked as its unplanned exaption is Hospital or extensive dental work. - Refur to Minimum Staffing Requirements to maintain operational requirement - Neg 4 hours EB8 a Award don't support S& Draft paper for leadership Team re: changing " ATO act to TOL - TOIL - P/T staff (PJ 27 18.5) GU, DW, PC & STA 07.09.2017. - ADR timeline incident with ARM 206. - Work Livingstons review -Ine - Problem with a process - Hug A - Workcover - PID - ESU notified AJR + Lawyer - Nov Work - Jan - meeting with JF + PC Jan- with JF 4 PID Ly past process is an issue -ESR - ESR review - No Straight answer. with Lawyers DW- seems that AJR Ke has an issue with the negatives so she can be a

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# **Paul Csoban**

From: Paul Csoban

Sent: Sunday, 24 September 2017 8:11 AM

To: Cathie Allen

**Subject:** Fwd: Amanda Reeves return to substantive position

Hi Cathie

Can we catch up on Monday am to discuss this please

Paul

Sent from my iPad

Begin forwarded message:

From: Gary Uhlmann <

Date: 22 September 2017 at 5:28:10 pm AEST

To: Paul Csoban < Cathie Allen <

Subject: FW: Amanda Reeves return to substantive position

Paul/Cathie – Please note my email to Amanda below. I am happy to discuss this in more detail next

week.

**Thanks** 

Gary



# **Gary Uhlmann**

Chief Executive Officer

Health Support Queensland, Department of Health

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From: Gary Uhlmann

Sent: Friday, 22 September 2017 5:27 PM

To:

**Cc:** Shaun Mulholland; Theresa Hodges; Dianne Woolley; Barbara Phillips **Subject:** Amanda Reeves return to substantive position

Hi Amanda

Thank you for your time this week. I was pleased to have the opportunity to explore and clarify your issues and concerns. I also noted your assurances that you support the current standing scientific processes and your additional confirmation that the processes currently in place also satisfy your concerns about the possibility of positive samples being missed although you do believe that there are more efficient ways of undertaking this testing.

I committed at that meeting I would finish my discussions and considerations about your current employment arrangements and operational concerns and notify you of my decisions at the end of this week.

As such I have decided the following:

- 1. That you should return to your substantive position within the DNA team on Tuesday 3 October 2017;
- 2. That the current process you believe is inefficient will be reviewed to determine whether it can be improved;
- 3. That a review will be undertaken to determine whether any previous negative samples should be retested to ensure that this negative result is accurate;
- 4. That upon your returning to your position, an external consultant will be engaged to help undertake the following reintegration activities:
  - a. Support the re-establishment of the management team and working relationships including clarification of any existing role, responsibilities and relationship issues and matters and obtaining a clear agreement on these matters by all parties.
  - b. Support the re-establishment of your individual team and address any matters that may impact on the effectiveness of your team.
  - c. Where a written commitment needs to be obtained between any of the parties in order to ensure the effective future operation of the overall DNA function then this will be completed as part of this process.

Please note that I consider that the effective operation of the DNA function is critical for the State and that the reintegration activities outlined above are an important step in ensuring the function is not compromised.

I will personally communicate the above decisions to FSS management to ensure everyone works in close partnership with the nominated consultant for the success of the implementation of the above decisions. My objective is that this work will begin on the 3<sup>rd</sup> October.

close partite is high with the normated consultant for the success of the implementation of the abo
decisions. My objective is that this work will begin on the 3 <sup>rd</sup> October.
Regards

Gary



# **Gary Uhlmann**

Chief Executive Officer

Health Support Queensland, Department of Health

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# **Cathie Allen**

From: Paul Csoban

Sent: Thursday, 11 January 2018 7:33 AM

To: Cathie Allen

Subject: FW: Draft Confidential Review Report

Attachments: FFS-DNA - Issues -Themes PM 100118 v2.pdf

Importance: High

Sensitivity: Confidential

From: Peter Mathews [mailto:

Sent: Wednesday, 10 January 2018 6:03 PM

To: Michel Lok; Paul Csoban

Cc: Paul Guyatt; Allan Holz; Allan Holz (Subject: Draft Confidential Review Report

Importance: High Sensitivity: Confidential

Michel and Paul

Attached is a draft report for consideration at our meeting at 0930 hours tomorrow morning.

I look forward to meeting with you.

Kind regards

Peter

# **Peter Mathews**

Managing Director



# **Cathie Allen**

From: Paul Csoban

Sent: Thursday, 11 January 2018 5:21 PM

To: Cathie Allen

**Subject:** FW: Action plan - change

Attachments: Schedule for Workplace Change - 11 January 2018.xls

FYI

From: Michel Lok

Sent: Thursday, 11 January 2018 4:58 PM

To: Paul Csoban

Subject: Action plan - change

Attached draft per our discussion this am.

Μ



# **Michel Lok**

General Manager

Strategy, Community and Scientific Support Health Support Queensland, **Department of Health** 

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#### Review - Forensic DNA Analysis Team Page 1 of 8

#### **Review - Forensic DNA Analysis Team**

#### **RELEVANT BACKGROUND**

Health Support Queensland (HSQ) businesses provide critical services to Hospital and Health Services, other government agencies, commercial clients and the community.

Within HSQ, Forensic and Scientific Services (FSS) delivers products and services in the areas of DNA profiling (forensic and non-forensic) and forensic chemistry, clinical forensic medicine, coronial services, and scientific services to support public and environmental health investigations.

Within the Police Services Stream, managed by Ms Cathie Allen, DNA Analysis and Reporting is undertaken by the Forensic DNA Analysis Team.

#### FORENSIC DNA ANALYSIS TEAM

The Forensic DNA Analysis Feam has seen a number of significant changes, both technical and operational, over at least the past decade, some of which have resulted in adjustments to the organisational structure.

These changes include the way that samples are received, changes in analytical procedures and technology and a recent change of information management system, from Auslab to Forensic Register.

The most significant development which contributed to the requirement for adjustments to operating procedures and organisational structure was the change from receiving evidence in the form of whole items to receiving evidentiary materials in tubes, after initial processing of the whole material by the Queensland Police Service (QPS).

The QPS then allocates a priority level to the sample which, effectively, acts as a measurable performance standard. F, for example, Forensic DNA Analysis has set standards for the following - Priority 1 samples are to be processed within 3 days, Priority 2 samples between 1 to 2 weeks and Priority 3 samples within 2 to 3 weeks.

This change in the evidence handling process enabled the Forensic DNA Analysis Team to arrange its Evidence Recovery and Analysis activities to operate as a throughput laboratory with sequential operations delivering results to reporting teams which then refine the information and generate the end product.

Consequently, the current organisational structure reflects a production line approach where materials are put through a refining process to produce an end-product that meets the requirements of customers.

From the information available, the last significant assessment of the organisational structure occurred in about 2008 and, given the number of significant changes that have occurred, since then, together with the recent history of significant interpersonal and operational dysfunction within the group, it is timely that the operating model is reviewed with a view to the revising the organisational structure

Organisational Structure @ 9 January 2018

#### **CURRENT SITUATION**

During the period since at least early to mid-2016, the Forensic DNA Analysis  $\pm$ team has been managing complex human resource issues that have adversely affected the operational efficiency and morale of the  $\pm$ team, at both the management and operational levels.

To assist FSS to better understand the basis for the ongoing operational dysfunction in the Forensic DNA Analysis <u>Team</u>, Workplace Edge has conducted interviews with senior management, the members of the management team and <u>operational</u> staff from the two Reporting Teams.

Contributing factors to the dysfunction in the Forensic DNA Analysis ‡team and the primary issues of concern to staff, identified in the course of these interviews, are shown below:

#### 1. Operations, Operating Model and supporting Structure

a) The production line model has not achieved the optimal delivery of services under the current structure with the existing systems and processes, and resource allocation.

It is not accurate to say that the production line model has not achieved the optimal delivery of services – as it had been delivering results which were noted as being the best in the country during the <u>national end toof</u> end evaluation undertaken by <u>the National Institute of Forensic Science</u> in 2011 and 2014. It is only in more recent times of change (<u>instrument issues and implementation of the FR</u>) and increased stress, that the end of the production line is not achieving the optimal delivery of results to the OPS.

b) The organisational structure does not fully support the current operating model as illustrated by comments provided by staff:

The Team, as a whole is over governed with 10 supervisors managing approximately 60 staff, giving a ratio of 1:6 actuals and between 1:4 and 1:5 FTE.

Staff members highlighted that the reporting teams were over-governed by supervisors and proposed that the two reporting teams could be merged into one team with one supervisor.

Commented [CJA1]: As there are Operational Officers within Forensic DNA Analysis, it would be better to remove the work 'operational' to reduce confusion.

Commented [CJA2]: Its my understanding that the comments made about over governed were restricted to the reporting teams, not the whole team. Its Workplace Edge's opinion that the whole team is over governed. This needs to be made clearer. The Analytical Line Manager oversees about 15 people, which I don't think is over governed.

- Projects take too long to establish and complete and there is no single point of accountability for bringing projects to completion.

Projects taking too long – the reporting staff interviewed were specifically discussing projects in which reporting were involved – this is a smaller proportion of projects than projects as a whole (ie discussing Y-STR project which is led by a Reporting Team Member as all bench work has been completed, as opposed to new instruments being implemented for use which has been completed in a shorter timeframe). Given staff are of a science background-and the-setatements are somewhat nonspecific, more detail is required.

The perception of the interviewed staff members that projects take too long may well be due to not receiving timely feedback and updates on the status of the projects from their line managers.

-The Quality and Projects, and Operational Officers Team provides Operational Support to the other Teams, but is not accountable for the delivery of projects and does not have a significant role in monitoring quality in the forensic reporting and intelligence teams. Combining the functions of Quality, Projects and operations support and placing this as a Team with a subgroup together with Evidence Recovery and Analysis has not met the organisational needs in the areas of Quality and Project management.

It is <u>not accurate incorrect</u> to say that <u>the Quality unit does not have a significant role in monitoring quality in the forensic reporting and intelligence teams. Dr Kirsten Scott provides significant input into SOPs, training modules and significant advice and guidance regarding quality to staff that see her. Quality is everyone's responsibility; therefore Justin Howes is responsible for operational quality from his team. Dr Kirsten Scott is responsible for overseeing that quality activities are undertaken appropriately <u>and she performs this function to a very high level</u>.</u>

It is <u>not accurate incorrect</u> to say that the function of Quality have not met the operational needs in the area of quality and projects – this team have ensured that a large number of projects have been completed, despite the delayed responses for feedback from the <u>some members of the management team.</u> Reporting team managers. It cannot be stated categorically that a team have not delivered, when the reasoning behind that is due to the delay from other staff members (regardless of the workload by the managers of the reporting teams, deadlines have been given and not met by them and not enforced due to working in an inclusive environment).

- The staff in the Reporting Teams are paid at a higher level than similarly qualified staff in the other teams, due to the presumption that they will be required to present results to the Courts and respond to examination by prosecution and defence attorneys. However, only around 10% of results are presented in Court and some members of the Reporting Teams may never, or only rarely, attend Court, which is seen by some as an inequity that contributes to disharmony in both the Reporting Teams and the Forensic DNA Analysis Team, as a whole.

It should be highlighted that staff who have never attend<u>ed</u> court are newer reporting team members who have not yet fulfilled their training and <u>have not</u> been deemed competent for court – it is <u>not accurate</u> incorrect to say 'never' and not provide detail surrounding this.

It is very difficult to ensure that all reporting team members attend court – given the case types may provide a plea, which is not the fault of the staff member. Ensuring that there is equal representation of staff in court can only be done when cases require re-allocation (which Justin does on an equity basis), however, court may still not go ahead and this is beyond of our control.

- The Production Line concept contributes to feelings of frustration among highly qualified staff, who would prefer to see less rigidity in the organisation and more equitable distribution of work.
- The Production Line concept has also led to the relative isolation of Teams. The restricted information sharing and limited professional interaction between staff has contributed to deskilling and re-work during the case management stage.

It is not accurate incorrect to say that the production line concept has led to the isolation of the teams – as this concept has been working extremely well for the most part since its inception. The reasoning behind it no longer working well is due to the culture that is currently within the management group, which has created mistrust and disharmony. leadership by the reporting managers, who undermine other managers during tea and lunch breaks to their team members. This causes disharmony. The reporting managers do not include themselves in group activities such as group morning teas, therefore staff members see this and therefore may not don't attend either. This is what contributes to the disharmony, not the production line process which has been demonstrated to be very successful by the NIFS end to end projects (twice).

Re-work during case management can also be due to lack of confidence by the staff member undertaking the work, which hasn't been highlighted.

Staff members have been advised that they are able to observe tasks being undertaken by the Evidence Recovery and Analytical teams, by liaison with the line managers of those teams.

- There is a management team comprising nine people who identify as managers for an overall compliment of around 70 people. It is large and unwieldy and has become dysfunctional, partly due to the interplay of particular personalities, but a contributing factor must also be its size and lack of internal structure and the expectations it generates.
- This dysfunction is evidenced by the failure to deliver projects and the failure to address critical issues such as the impending technical changes to DNA Aanalysis for intelligence purposes, the breakdown of the Intelligence Team and the failure to manage to bottleneck in the production line between Analytical and Reporting.

It is not accurate to include the breakdown of the Intelligence Team as a dysfunction of the management team. During the FR project, it was highlighted that changes would be required within

this team, however a gentle approach to this change was taken. Personal issues within this team have not been included in this review as those staff members were not interviewed.

It is not accurate to place the failure of managing the bottleneck of results onto the management team when members of this team are ensuring that results are being reported, on top of the other tasks that they currently perform. This is a more complex issue that requires more detail than what has been provided.

It is not warranted placing all of the blame of the bottleneck of results onto the management team—they have all provided advice and ideas on how to decrease the number of outstanding results (including Allan and Luke interpreting hundreds of results on top of their work), when it is largely a failure of the management members within the Reporting teams to act upon the issues

- Within the Reporting Teams the piecemeal basis on which work is allocated contributes to inefficiencies, particularly an overall low work output, inequitable sharing of the workload and low levels of reported work satisfaction.

#### AGREED

#### 2. Culture

- a) Whole Group Issues
- There has been a failure, over the long term, to effectively address human resource management issues so that by the end of 2017; despite repeated attempts by senior managers: o ordinary line management reporting was not in operation between the Reporting Teams and the Managing Scientist;
- o the management team was not functioning effectively, due to an undercurrent of personal disagreements;
  - o a number of personal grievances remained unresolved; and
  - o the Intelligence Team was without an effective compliment of staff.
- Vertically and horizontally, within the Forensic DNA Analysis team, there are significant communication issues. It is perceived that there is inadequate communication by senior managers, which contributes to the circulation of pernicious rumours.
- There is a perceived lack of transparency in decision making, which contributes to high levels of suspicion and separation into cliques, with the resultant breakdown in trust amongst staff and management.

The perceived lack of transparency in decision making is also due to the circulation of the pernicious rumours from <u>some staff members</u> reporting management staff and their undermining of other management team members – this <u>hasn't</u> been detailed effectively in the above statement. <del>doesn't</del> seem to have been adequately addressed

- There are significant issues regarding priorities for action, where personal relationship and grievance issues have distracted management from priority operational decisions. This has resulted in inefficiencies and a failure to resolve bottlenecks with a resultant perceived impact on customer service.
- There is a breakdown in line management processes and respect for normal workplace behaviour, as a result of the failure to apply ordinary performance standards in relation to conduct and professional output.
- There is a lack of flexibility in leadership and management to address operational performance issues and to adjust the operating model and allocation of resources to address these issues.

It is not accurate to detail the above as it is because it doesn't describe the above as being statements from staff members, so it is their perception of the situation.

- Morale is low, and the reporting list is growing without any clear plan to reduce the list. This is very upsetting for all of the Reporting staff. Overtime is a short-term solution to a long-term problem.

The statement 'This is very upsetting for all of the reporting staff' would be extended to 'all staff members', as all team members are affected by the <u>decreased lack of</u> output from the reporting teams.

Similarly "the reporting list is growing without any clear plan to reduce the list" is <u>not accurate</u> incorrect. This issue has been the subject of discussion amongst senior management and there are plans to utilise FR and <u>planned overtime activities</u> mooted restructures to <u>assist in</u> addressing this situation.

- b) Reporting and Intelligence Team Issues
- The Reporting function is over-governed with two supervisors when in effect, it operates as a single team.

- The system of work allocation is inefficient, with reported co-dependence of the supervisors, resulting in inequitable piecemeal allocation of work.

#### The above statement is how the reporting teams describe their work environment.

- There is a lack of appropriate performance standards and monitoring, with a resultant lack of accountability for individual performance, which contributes to less than optimal production outcomes. This contributes to a lack of job satisfaction, and concern by individuals at the performance variability amongst staff.

#### The above statement is how the reporting teams described their work environment.

- The Reporting Team comprises staff with a wide variety of skills qualifications and experience, which is not fully utilised due to the rigidity of the production line operating model. This has created silos, with little opportunity for staff to broaden the use of their skills and experience.

It has also been stated that a further contribution to staff being unable to broaden their use of skills and experience <u>is</u> due to favouritism by the reporting managers – i.e. only particular staff are allocated 'projects' to undertake and that this is unfairly distributed – this doesn't seem to have been included when it should be considered as a major factor.

- There is considerable re-work when a particular case is received by the Reporting Team, and full consideration is given to the evidentiary issues. This is primarily due to a lack of consultation across silos, as the case progresses through the Evidence Recovery and Analytical areas.

The re-working of some samples that can be undertaken by a reporting staff member can be due to additional reasons – e.g. additional information has been received that was not available when the items arrived which requiring rework, etc. There is an apparent lack of trust from the senior staff members within the reporting teams regarding the work undertaken by the Evidence Recovery and Analytical teams – this contributes to the reworking undertaken, however this hasn't been highlighted. The reporting managers openly display a lack of trust in the work undertaken by the ER and Analytical teams and this facet has been taken on board by reporting staff members – this can be considered as a major contributing factor.

- The Intelligence Team has virtually ceased to operate due to the loss of an effective compliment of staff.

The loss of an effective compliment of staff from the Intel team is beyond management control – however and plans are being implemented to address this.

- This Unit currently uses the nine loci DNA kit, which is no longer in commercial production, and there is a requirement for new business rules to be developed with QPS to support the use of the 21 loci DNA kit for intelligence purposes.

It should be noted that **Volume Crime** items are processed with the 9 loci DNA kit and that these items now need to be processed in another kit – which <u>the QPS</u> has deemed to be <u>the PP21 kit</u>. The statement about the 'Unit using 9 loci' is very unclear around content and relevance.

- The Intelligence Team is responsible for uploads to the national data base, which is not the most efficient allocation of this task. There is merit in considering merging of Intelligence with Reporting and then training the integrated team to perform uploads.

It is not an effective use of HP4 reporter's time in uploading of profiles to NCIDD. Whilst the task of reviewing links can be done by HP4 reporting staff (given they report other similar results), it is not envisaged that HP4 reporters should upload profiles to NCIDD. This statement is too simplistic in its current wording. In the process of integrating the Intel team, the tasks are planned to be broken down and reallocated to appropriate HP levels.

#### 3. Systems and Processes - Forensic Register (FR)

The introduction of FR is an opportunity to review systems and processes, particularly to inform the further development of FR. Whilst the new system has not been fully implemented at this stage, it will result in changes to work processes and work practices and ultimately structure and resourcing. Any immediate changes from this review need to take into account that further adjustments may be necessary as FR is fully implemented.

The FR has been fully implemented – it is <u>not accurate incorrect</u> to say that it hasn't. <u>FSSWe</u> have reported to all (including within the QPS) that <u>the FR has been</u> <u>we have</u> implemented the FR. There are elements to the reporting processes that require enhancement, however the statement as it stands is <u>not accurate-incorrect</u> and contradicts the reporting that we have previously submitted for the Project (FRIP). It should be noted that the Project team for this implement<u>ation ed</u> has in fact been disbanded due to completion.

The reporting teams have been incorrectly advised that certain parts of their processes weren't addressed. A large amount of <u>development</u> work was <u>directed to ent into</u> the automatic reporting lines within the FR, however the reporting matrix provided to the QPS had a large number of errors in it which caused wrong lines to be provided. This was a large risk for both organisations, so with the agreement of the Team Leaders, this portion was postponed until after implementation. This meant that the system of choosing a line that is done in Auslab was done in the FR until further work could be done to correct the matrix. Progress has been made on this and the reporting staff are giving feedback on the automated lines that are now available in the FR. It is due to this miscommunication regarding the FR that staff have built further false impressions.

#### 4. Conditions of Employment

It has been raised that part-time staff are not allowed to accumulate TOIL and that this is a blanket ban which is not applied to other FSS staff. It is reported that, part-time staff in Forensic Chemistry do have access to TOIL.

Forensic Chemistry staff work under a different arrangement – staff voted for a Variable working arrangement meaning the accumulation of ATO. This has been communicated on numerous occasions. Forensic DNA Analysis voted for a Standard arrangement, meaning the accumulation of

TOIL. Under the Award arrangements, part-time staff were only able to accumulate TOIL after they had completed an 8 hour shift. Due to budget constraints, it was not feasible to allow staff to work additional hours and accumulate TOIL, unless required for urgent items or court. Clarification has been sought from HR regarding the new Award and Enterprise Bargain Agreement.

Staff have put the view that denial of access to TOIL and the rigidity applied to 'spread of hours' affects flexibility in the workplace and personal wellbeing and is not consistent with the family friendly policies of the Department.

The spread of hours is due to the <u>service that is provided to our clients, namely the QPS and the Court reporting staff being required for Court</u>—which is open between 9am and 5pm. Notification of <u>the requirement for</u> evidence is not always supplied <u>in advance</u>. Thus if we accept flexibility according to some staff wishes, the situation could arise where all staff have left at 2.30pm (due to a 6am start) and court <u>evidence</u> is required at 3pm. <u>This would not be providing a service to the client.</u>

This statement also hasn't been balanced with the <u>client's request for availability during court</u> <u>business hour</u> <u>fact that we are required by the client during business hours</u> (the QPS 8am to 4pm; the courts 9am to 5pm). It should be noted that this has already been the subject of union negotiation and in fact has been settled.

#### 5. Training & Development

Staff reported that there were few opportunities to gain broader experience in other roles and that limited training opportunities exacerbate this problem.

Training is ad hoc and restricted to the work staff are undertaking in their substantive role. It is poorly organised and not needs-based.

There are limited opportunities for teams to share what they are undertaking and to learn from each other.

A reporting staff member attends the Evidence Recovery team meeting and a different reporting staff member attends the Analytical team meeting – feedback should be provided from these team members at their meetings. As the reporting teams don't hold meetings, there is no mechanism for feedback and this hasn't been highlighted.

<u>Due to the requirement for the provision of timely results to the client, training outside their core duties is not able to be provided.</u> A training matrix is currently being developed in the reporting teams to ensure that all staff are trained across all tasks required.

#### RECOMMENDATIONS

1. Operating Model and Structural Options

**Option 1: Process Integrated Team Approach** 

This option would involve a shift from the production line model to integrated horizontal teams which would handle cases through all process stages. The model would split the teams horizontally into product segments, for example:

- 1. Major crime;
- 2. Sexual assault; and
- 3. Volume crime.

There are some significant benefits in adopting this model, as follows:

- 1. Reduction of silos;
- 2. More flexible working arrangements;
- 3. Greater variety of work for individual staff;
- 4. Increased skills and experience development opportunities for staff;
- 5. An opportunity to develop a more collaborative and cooperative team-based operating approach, which would increase the flexibility to allocate resources where the greatest demand for work was located, and to speedily remove any blockages such as in the reporting area; and
- 6. Potentially less re-work which currently occurs under the production line model due to the siloed nature of work.

#### Implications:

- 1. Three new Team Leaders at HP6 levels to lead the teams.
- 2. Reduction in Supervisors -5 x HP5's (Evidence, Analytical, Reporting x 2, Intelligence x 1) and 1 x HP4 (Operations).
- 3. Reduction in two Team Leaders 2 x HP6 (may be successful in the three new Team Leader positions)
- 4. Possible shift in resources to strengthen Quality and Projects Unit.

It is inefficient and not cost effective to have 3 Analytical teams – given the large number of volume crimes samples are required to there is a requirement to ensure a timely throughput of major crime samples (ie larger batches enable more samples to be processed efficiently). The proposed structure is not viable given workload and resource constraints.

In fact this may create more silos, as the ER staff wouldn't see the variety of samples that they currently have access to.

#### **Option 2: Enhanced Production Line Model**

This option would involve structural and process changes to address many of the concerns expressed above.

The proposed changes to the current model would involve:

- 1. Separating the Quality and Projects functions from Operational Support. Having this function report directly under the Managing Scientist will provide an overarching service to all program activities and units.
- 2. Merging the two reporting teams into one unit and also merging the Intelligence Team into the merged Reporting Team.
- 3. Reducing the size of the management team to four positions namely: a. Managing Scientist  $\,$ 
  - b. Quality and Projects Manager
  - c. Team Leader Evidence Recovery and Quality;
  - d. Team Leader Forensic Reporting and Intelligence
- 4. Establishing a Technical Advisory Group (or Reference Group), which would comprise Supervisors in Evidence Recovery, Analytical, Reporting, Operations and Quality/Projects and other staff on as as-needs basis, depending on the nature of the technical, scientific or operations matter for consideration.

#### Implications:

- 1. Reduction in supervisors  $-3 \times HP5$  these positions may take up other roles or take up the duties of the area on a (Present Incumbent Only) PIO basis.
- 2. Quality and Projects is elevated to a whole of team oversight and support position reporting directly to the Chief Scientist.

There seems to be a fundamental misunderstanding regarding Quality and Projects – this position previously reported directly to the Managing Scientist, however this did not change the perception of the role. The fundamental piece that is missing is that some staff members the reporting managers delay projects and do not provide feedback to their teams on projects. If this were adequately addressed, the perception of Quality and projects would change.

Note: There is no Chief Scientist - presume this is the Managing Scientist

#### **SUMMARY RECOMMENDATIONS**

REC 1. Operational Model - consider the options for operational model and structural change, and assess the merits of each option and the implications, and decide which is the most appropriate option. Should Option 2 be the most appropriate option, consider the establishment of a Technical Advisory Group (TAG) or Reference Group, the function of which would be to support decision making at the technical and operational issue level. This Group would comprise Senior Team Leaders and Supervisors and others on an as-needs basis. This Group would not usurp the role of management, but rather address operational and technical issues and provide advice to the management team, thus freeing up the management team to address strategic issues.

Changing the name from Management Team to Technical Advisory Group wouldn't seem to assist with the issues that are being considered for resolution

REC 2. **Quality and Projects** - notwithstanding the choice of operating model, it is recommended that Quality and Projects is strengthened with additional resources, and reports directly to the Managing Scientist, to enable the Unit to provide overarching quality review and project delivery across the whole business.

This is feasible but care should be taken that by placing Quality & projects under the Managing Scientist, the expectation would be that the Managing Scientist is able to achieve more than others do currently. The change would have to be framed appropriately and may be seen as reverting to old ways.

REC 3. **Court attendance** – review the officers currently qualified to attend court and undertake an assessment of the need for court attendance, the number of staff required for this function, and the most appropriate staff members to attend court. This may involve additional training and development for some officers.

This has ramifications for Workforce Planning. The proposal means that some staff would retain their HP4 role but not attend court – this is very difficult to justify. Given the large number of items waiting reporting, supporting this would have implications for workflow, given it is beyond our control to know or estimate court requirements

REC 4. **Performance Framework** – develop and implement an appropriate performance framework and system with clear standards for operational delivery and throughput for each position. Ensure there is alignment of expectations between staff and managers/supervisors, and that staff are regularly assessed and coached against agreed KPIs and performance criteria. This will ensure equitable allocation and delivery of work.

#### Agree

REC 5. **Bottlenecks** – with whichever model is chosen, and whatever recommendations are adopted, ensure that managers and supervisors identify fluctuations in capability and capacity to deliver in certain areas early, and develop appropriate strategies to address the shortfall speedily prior to the gap becoming a major problem.

This recommendation should include a requirement that PDPs for the reporting managers should note responsibility for responding to the changing needs. There is no bottleneck at the front end of

the process as the line managers currently take responsibility and accountability for the work and put adequate processes in place.

REC 6. **HR Issues** – ensure the appointment of an HR Manager and supporting resources at FSS to provide on time HR advice, and support, including advice on process and strategic interventions. Ensure that all HR issues are triaged, and addressed equitably and promptly to avoid escalation and dysfunction in the organisation.

#### Strongly Agree

REC 7. **Communication** – develop an internal communications strategy based on the communications issues identified, and ensure that the strategies are implemented using approaches such as more regular team meetings, timely communication of decisions impacting staff, internal communiques, intranet posts, management "walking the talk" and other targeted strategies and actions.

#### Agree

REC 8. **Systems and Processes** – ensure there are appropriate processes in place to support the implementation of FR, and ensure that internal systems and processes are developed to ensure staff utilise the full capacity and capability of FR, which may result in streamlining of workflows and an increase in productivity.

FR is already implemented; there is a structure in place that supports <u>ongoing</u> enhancements (VSTS, fortnightly meetings, changes rolled out and SOPs changed). An FAQ is currently being drafted.

REC 9. **Conditions of employment** – review the conditions of employment in areas such as TOIL, and rigidity in the spread of hours, and ensure that staff are treated equitably across different professional, operational and administrative areas.

Staff are all treated equitably according to the operational needs of the unit as outlined above. Additionally there is a current <u>request investigation</u> underway around the latest Award and EB provisions.

REC 10. **Utilisation of skills and experience base** – depending on the operating model chosen, ensure that all staff have an equitable opportunity to undertake work and duties in areas where their skills and experience can be applied, and they have opportunities to enhance their skills through targeted training and development.

# Agree

REC 11. **Outstanding operational issues** – ensure that processes are in place to address outstanding operational changes, and that staff are up to date with the latest techniques and approaches eg change from nine loci DNA kits to 21 loci DNA kits.

#### Agree

# **Cathie Allen**

From: Cathie Allen

Sent: Thursday, 25 January 2018 4:10 PM

To: Paul Csoban;
Subject: Feedback

#### Hi Paul & Allan

Justin has provided me with some feedback after a discussion with Kylie Rika. Kylie advised:

- At least half the staff have said that the presentation didn't represent their views or what they provided in the interviews
- Staff have openly discussed this in their work area and the vocal staff who said it wasn't their view noted the staff who didn't join in the conversation
- It was put forward that Allan, Cathie and Paul only put forward the things that they wanted to say and not the views of the staff
- Kylie has questioned the integrity of Workplace Edge by saying that they are paid for by QH, so that adds weight to the view that it was QH's views put forward and not the staff members

I'm concerned about this type of discussion and the affect that it will have on the staff member who have opened up. And I would welcome any ideas on what we could do to quell some of this nonsense.

# Cheers Cathie

# 7

#### Cathie Allen

Managing Scientist - Police Services Stream

Forensic & Scientific Services, Health Support Queensland, **Department of Health** 

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# HealthSupport Queensland Forensic and Scientific Services

Summary of feedback from Reporting Teams – Forensic DNA Analysis, after interviews conducted by Workplace Edge

A significant number of staff expressed the following perceptions:

- The morale within the reporting teams is low
- There is a perceived lack of accountability for work output and performance is not actively managed.
- There is a perception that with an increasing workload, there is no clear plan to manage it.
- The Commonwealth Games will be held shortly and there has been no plan communicated to the teams regarding this event.
- Communication needs to be improved between vertical levels of management.
- There is a perception that gossip is damaging and there is a failure to manage pernicious rumours
- There is a perception that the separation between ER & Q teams and FRIT was a result of the development of antagonistic attitudes, which was further entrenched by a lack of socialising and reduction in other meaningful interactions.

# Way Forward

- FRIT management will investigate ways to improve the interactions between the teams to improve information sharing and ensure shared decision making where this is appropriate
- A culture development process will be undertaken to address the low morale within the teams along with other areas identified
- Management Team will be looking at the best ways to achieve improved communications and more timely action on problems that affect your well-being.
- Internal processes will be open, merit based and transparent in accordance with Departmental policies. The communications regarding these internal processes will also enable and demonstrate this.
- A key goal through the cultural development process will be to ensure a culture of inclusion and a shared sense of achievement.
- FSS are examining options for improving HR and IR support for the campus
- Increasing the availability of HR resources will assist in addressing issues regarding improper conduct in a timely manner
- Further consultation with all staff of Forensic DNA Analysis will be undertaken in light of the feedback regarding team organisation and team functioning
- Suggestion regarding training needs for staff and the benefits from widening participation in the use of Moot Courts and other training methods will be considered
- Frequently Asked Questions regarding Forensic Register has been provided to all staff members in Police Services Stream
- Further consultation and consideration given to the large amount of feedback regarding quality and projects.
- Workshops to be undertaken regarding the functioning of the individual reporting teams.
- Workflow devised regarding volume crime samples being profiled with PP21
- Commonwealth Games is anticipated to increase the number of items submitted and workforce management plans have been implemented for this period.

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# **Cathie Allen**

From: Paul Csoban

Sent: Thursday, 1 February 2018 7:58 AM

To: Cathie Allen;

Subject: RE: Summary doc

#### Hi Cathie

I think that sums it up well

Regards Paul

From: Cathie Allen

Sent: Wednesday, 31 January 2018 2:10 PM

To: Paul Csoban; Subject: Summary doc

Hi Paul and Allan

A couple of staff members from the reporting teams have requested a copy of the presentation. The presentation is not sufficient on its own to tell the story as it requires the words that we wrapped around it. I've put together a Summary document that I thought we could give to staff and would like your feedback. Whilst I have minimised the feedback, I'm attempting to focus on the way forward. Please let me know if I've minimised too much and I can add more in.

# Cheers Cathie



### Cathie Allen

Managing Scientist - Police Services Stream

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# Additional information

Tolli. Callie Alleli	From:	Cathie Allen <
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To: Ashley Macfarlane <

Date: Fri, 23 Mar 2018 17:28:05 +1100

Hi Ashley

Thank you for your me y esterday. I'd like to provide the below informaon, in addion t o the informaon provided yesterday.

Addional clarific aon r egarding Selecon R eport for Luke Ryan:

Three candidates that v	were shortlisted and offered interviews had (	extensi <u>ve forens</u>	<u>sic experience, how</u> ev	er each of then
were currently <u>w</u>	the forensic field. These candidates		a	nd Luke Ryan.
One candidate,	, did not proceed with the interview	v, as sh <mark>e</mark>		w that she had
accepted a forensic pos	sion in the Northern T erritory forensic DNA	laboratory. It w		
	eir current roles were not in a forensic area,			
	to show transparency for their inclusion		es to me that the No	
	o used a similar inclusionary process, as		sful in gaining a posic	on within the
forensic area despite h	er current posion being outside the forensi	c field.		

Insp Brendan Smith was known to me through professional meengs on coronial malers, however I did not know Insp Smith on a personal level. Whilst I have come to know a number of police officers on a personal level through my alendance at forensic work meengs and forensic science conferences, I didn't know Insp Smith on a personal level prior to the recruitment process. I have not socialised with Insp Smith, either before the recruitment process or since the process was finalised. To my knowledge, none of the applicants knew Insp Smith prior to the recruitment process and it was for this reason that Insp Smith made an ideal, independent panel member. Insp Smith was not surprised by my declaraon of knowledge of several candidates, as through the shortlisn process he was aware of their current or previous employment at FSS as it was listed on their CVs.

Addional in formaon r egarding social interacons:

The Queensland Police Service hold Christmas pares and some mes, s taff members from Forensic DNA Analysis were invited and a ended some of those pares. A tone Christmas party, which was held at Boggo Road Goal, it was a ended by myself and several other work colleagues which included Luke Ryan and Amanda Reeves (this was in approximately 2004). This is to highlight that over the many years of different social funcons held, a v ariety of work colleagues have socialised with Luke Ryan, not just myself.

I would like to place on record that I consider this complaint lodged against me to be retribuon and vexaous for perceived issues regarding Amanda Reeves. In June 2016, Amanda has a negave interacon with another member of the Forensic DNA Analysis management team, Allan McNevin. This interacon occurr ed whilst I was on leave and upon my return, I was updated with the status of the situaon, which was that the issue between the 2 pares hadn't been resolved and that Amanda had lodged an email with Allan's line manager detailing 4 allegaons ag ainst him. These allegaons hadn't been in vesg ated or resolved. I provided opons to Paul Csoban regarding potenal a venues to resolve the allegaons. P aul opted for an external invesg aon b y Livingstones, which was approved by Gary Uhlmann, then HSQ CEO. This external invesg aon c ommenced in approx. October 2016. In November 2016, Amanda insg ated a WorkCover claim. In late January 2017, Amanda was able to return to the work unit with full medical clearance, however she raised an issue with a process that was undertaken by the Evidence Recovery team, which is overseen by Allan. She advised that she would be unable to report on a category of case types due to this issue. The organisaon requested Amanda to undertake a project role at FSS whilst an audit was conducted on the process, this was approved and signed off by Gary Uhlmann. The audit by an overseas company showed that there was no issue with the process. Since Amanda's return to the FSS campus in Jan 2017, she has either ignored me when we passed each other in the corridor despite me politely speaking to her, or she has barely made an audible sound towards me, she had lodged a complaint against me with Gary Uhlmann in June 2017 (which to my knowledge remains unresolved as I have not been formally advised of this complaint), she has lodged four RTIs regarding material that I (and others) may hold that highlights my alleged a empts to remove her from her posion, she has c ontacted many HSQ staff members and spoken negav ely about me and she has spoken negav ely about me to two external consultants - Angela Pee (from Workplace Consultant) and Allan Holz (from Workplace Edge). For all of these reasons, I feel that this complaint is retribuon f or a perceived acon tha til have allegedly taken against Amanda and I feel that this complaint is vexaous. Amanda has also lodged complaints against other FSS staff members including Paul Csoban.

Further emails will be sent with a achments from the interview process.

If you need any clarificaon, please don't hesit ate to contact me.

#### Cheers Cathie



# **Cathie Allen**

Managing Scientist - Police Services Stream

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