Project #181 Spermatozoa Microscopy Sensitivity - Report

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Project Proposal #181 - Investigation into the sensitivity of spermatozoa microscopy

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Contents

1. Abstract

This project was undertaken following observations from staff where on occasions differences 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 improved.

The initial aim of the project was to investigate the performance of the current ER (Evidence Recovery Team) microscopy slide preparation process, in terms of relative sensitivity for spermatozoa detection and presumptive testing for seminal fluid (Acid Phosphatase (AP) and p30).

An attempt was made to develop a novel replacement ER process with improved sensitivity in spermatozoa microscopy. Next, a proposal that sexual assault swabs may be submitted straight for Differential Lysis (Diff Lysis), without first performing microscopy at ER examination was explored. This proposed method was adapted to preserve the ability to conduct presumptive testing. Performance of the 'proposed method' was compared to the 'current method', and further work was conducted to optimise the new process. Experimental variables were expanded to ensure that the proposed method was suitable for use with a range of sample substrate types, semen donors and sampling methods, to emulate casework.

Results showed the proposed method performed favourably to current ER microscopy across a range of variables. The proposed method gave mostly comparable detection sensitivity to current methods for p30 and Phadebas presumptive testing. Notably, the detection sensitivity for AP was slightly better for the current method than the proposed method. The sensitivity of p30 was shown to be superior to that of AP across most experiments.

It was concluded that the proposed method is suitable for laboratory requirements and is recommended to be introduced for standard processing of casework samples for semen testing, to replace current methodology. An additional recommendation was made, that p30 testing alone be utilised for seminal fluid presumptive screening, with AP testing capacity being retained solely for the purpose of locating potential semen stains on whole items.

2. Introduction

2.1. Background Information

In 2015, a small number of sexual assault casework samples were observed to 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.

Within Forensic DNA Analysis, spermatozoa numbers are graded during microscopy using a semi-quantitative scale:

0	(0)	None seen
<+	(<1+)	Very hard to find (Use England Finder Graticule)
+	(1+)	Hard to find
++	(2+)	Easy to find
+++	(3+)	Very easy to find
++++	(4+)	Abundant

ER microscopy differentiates between whole spermatozoa (a sperm head with attached tail), and sperm heads alone, without tails. Following Diff Lysis, whole sperm are not typically seen and therefore, at Diff Lysis microscopy only sperm heads are counted.

Isolated examples were highlighted from casework where 0 or <1+ spermatozoa had been observed during ER microscopy, yet when viewing the DNA profiling results obtained, there was evidently sufficient male DNA present within the sample to produce a strong contribution of male DNA (well amplified peaks, including an Amelogenin 'X,Y' genotype). These unexpected findings were investigated by preparing a second microscopy slide from the remaining Sperm Lysate produced during Diff Lysis Extraction. When these Diff Lysis slides were viewed microscopically, a large increase in the numbers of spermatozoa observed was sometimes seen, some with observations of 3+ or 4+ sperm heads. The comparative differences between the performance of the two microscopy processes were considered significant enough to prompt further investigation.

A moderate increase in the concentration of spermatozoa from ER microscopy to Diff Lysis microscopy is not unexpected, due to the sample liquid volume decreasing from ~200 µL to <50 µL during standard laboratory processing. A significant comparative reduction in spermatozoa microscopy count for the ER slide is not desirable however and may suggest limitations to the effective sensitivity of ER microscopy as an initial screening test for the presence of semen.

The observation of spermatozoa during microscopy is reported as confirmatory for the presence of semen detected within a sample, and the presence of a single

spermatozoon is considered sufficient in this regard. This information may potentially be critical for the client, depending on the nature of the allegation under investigation.

Due to the requirement for microscopy to be performed on a sub-sample taken from a larger volume of original material, it is impossible to know whether a 'negative' result may truly be representative of the whole sample, or instead simply due to having sampled a portion which does not contain any of the spermatozoa that may still be present in the remaining sample volume. This is sometimes referred to as stochastic effects. 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 more than one sub-sample is collected from a larger item, thus somewhat reducing the overall risk to case.

2.2. Previous investigations

Data Analysis was conducted on 79 casework samples processed through both ER microscopy and Diff Lysis microscopy between 2014 and 2016. Sperm Microscopy results, DNA quantification values of the sperm lysates and DNA profiling results obtained were compared and analysed.

Results of this analysis are recorded in:

I:\Change Management\Proposal#181 - Sperm microscopy sensitivity\Previous studies etc\2016 comparison of original v diff micro

In summary, the data indicated:

52 samples with a higher sperm count observed on the Diff Lysis slide;

10 samples with a higher sperm count observed on the ER slide;

17 samples with concordant sperm counts observed on each slide.

Overall there was a consistent trend towards improved spermatozoa microscopy sensitivity observed for the Diff Lysis slide, in comparison to the ER slide. The average quantification values from sperm lysates were observed to correlate well with sperm counts obtained from Diff Lysis slide microscopy but correlate less well with initial ER microscopy. Large differences between the two sperm counts were not observed within the analysed dataset.

These results prompted the proposal of Project #181 in order to conduct further investigations. This progressively developed into a multi-stage project, based on assessment of the experimental data obtained at each stage. During the project, different experiments were conducted employing various naming conventions. For clarity this final report refers to each experiment numerically (Parts 1 - 7).

Note: Prior to Project #181, microscopy results and / or presumptive tests for seminal fluid (AP and p30), were used to direct the onward workflow of sexual assault casework samples. A positive result for semen/seminal fluid would direct a sample to undergo Diff Lysis extraction, whereas a negative result may lead to a standard cellular extraction or cessation of testing depending on the strategy employed. Due to the potential risk of an insufficiently sensitive ER microscopy process, a risk mitigation strategy was implemented for the duration of Project #181. Any "Query semen" casework samples observed to be spermatozoa negative at ER examination microscopy underwent Diff Lysis Extraction and subsequent microscopy. The final sperm microscopy result reported back to the Queensland Police Service (QPS) would be based upon the final result obtained from the Diff Lysis slide. This measure was intended to prevent any samples containing low levels of semen being inadvertently treated as 'semen negative'.

3. Governance

3.1. Project Personnel

Project Manager: Allan McNevin, Senior Scientist, Evidence Recovery Team

Project Officers: Chelsea Savage, Scientist, Quality and Projects Team; Emma Caunt, Scientist, Forensic Reporting and Intelligence Team; Matthew Hunt, Scientist, Forensic Reporting and Intelligence Team

3.2. Decision-Making Group

The Management Team and the Project Manager formed the Decision-Making Group for this project.

4. Resources

The following resources were utilised for this project:

4.1. Reagents

- 5% v/v Bleach White N Bright (Ecolab, NSW, AU)
- 5% v/v Trigene Advance (CEVA DEIVET Pty. Ltd. Seven Hills, NSW, AU)
- Ethanol (Recochem Incorporated, Wynnum, QLD, AU)
- Nanopure water (Forensic DNA Analysis, Brisbane, QLD, AU)
- Proteinase K (20mg/mL) (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Brentamine Fast blue B (Sigma Aldrich, Castle Hill, NSW, Australia)
- Anhydrous Sodium Acetate (Sigma Aldrich, Castle Hill, NSW, Australia)
- Glacial acetic acid (Univar AJAX Finechem Pty. Ltd., Taren Point, NSW, Australia)
- Sodium α-naphthyl phosphate (Sigma Aldrich, Castle Hill, NSW, Australia)
- Sodium hypochlorite (10% bleach solution) (LabCo)

- Sodium sulphite (Sigma Aldrich)
- Nanopure water (Millipore Milli-Q® Advantage A10 system)
- ABA card p30 test kits (Abacus Diagnostics)
- Haematoxylin and Eosin stains (Forensic DNA Analysis, Brisbane, QLD, AU)
- Dithiothreitol (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Sarcosyl (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Positive controls (Forensic DNA Analysis, Brisbane, QLD, AU)
- TNE (Forensic DNA Analysis, Brisbane, QLD, AU)
- Phadebas® Amylase test tablets (Phadebas, Kristianstad, Sweden)
- Pertex® Mounting medium (Medite)

4.2. Materials

- Sterile 1.5 mL and 2 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- Sterile 5 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- ART Filtered 1000 µL, 300 µL & 20p pipette tips (Molecular BioProducts Inc., San Diego, CA, US)
- F1-ClipTip pipette tips - 20µL, 50µL, 200µL & 1000 µL (Thermo Fisher Scientific Inc,)
- Nunc™ Bank-It™ tubes (Nunc A/S DK-4000 Roskilde, Denmark)
- Rediwipes (Cello Paper Pty. Ltd., Fairfield, NSW, AU)
- Petri dishes (Starstedt Australia Pty. Ltd., Mawson Lakes, SA, AU)
- Sterile rayon swabs (Copan Diagnostics Inc., Murrieta, CA, USA)
- Sterile cotton swabs (Biomerieux, Norwest, NSW, AU)
- Grale HDS SureFrost™ Microscope slides (Trajan Scientific, Milton Keynes, United Kingdom)
- Coverslips (Menzel-Glaser)
- 1000 mL Schott glass bottles (Schott Duran®)
- Glass beakers (various)
- Adhesive tape (various)
- Paper bags (various)
- Permanent felt-tip markers (various)
- Highlighter pens (various)
- Plain white cotton t-shirts (Kmart, Australia)
- White cotton mix towels (Kmart, Australia)

4.3. Equipment

- Biological safety cabinets class II (ESCO, Lytton, QLD, AU)
- Vortex Mixer VM1 (Ratek Instruments Pty Ltd, Melbourne, VIC, AU)
- MixMate (Eppendorf AG, Hamburg, DE)
- Micro centrifuge (Tomy, Tokyo, JP)
- Eppendorf 5424 centrifuge and Eppendorf 5804 centrifuge (Eppendorf, North Ryde, NSW, Australia)
- Dry Block Heater (Ratek, Boronia, NSW, Australia)
- Thermomixer (Eppendorf, Hamburg, DE and Thermo Fisher Scientific (Finnpipette), Waltham, MA, US)
- FSE Waterbath (Nickel-Electro Ltd., Weston-Super-Mare, UK)
- Milli-Q® Integral 3 (A10) System with Q-POD™ (Millipore™, Billerica, MA, USA)

- Pipettes (Eppendorf, Hamburg, DE and Thermo Fisher Scientific (Finnpipette), Waltham, MA, US)
- ClipTip Pipettes (Thermoscientific)
- Promega Maxwell® 16 MDx 1 and 2 Instruments (Promega Corp., Madison, WI, USA)
- Milli-Q® Integral 3 (A10) System with Q-POD™ (Millipore™, Billerica, MA, US)
- Minifuge (CS Bio Co. (ex-Tomy Tech US Inc.), Menlo Park, CA, US)
- Tube Centrifuge (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)
- BX41 Microscope (Olympus Corporation, Tokyo, Japan)
- Mixers (various)
- Magnetic stirring sticks (various)
- Tweezers (various)
- Timers (various)
- Drying line and pegs (various)

Forensic DNA Analysis Evidence Recovery staff, computer and instrument time, as well as bench space in Forensic DNA Analysis Evidence Recovery Laboratory were also used during this project.

5. Part 1

5.1. Part 1 - Purpose and Scope

To investigate the sensitivity of the ER slide microscopy process, two questions are posed:

I. Was the ER slide staining procedure performing sub-optimally, such that spermatozoa are potentially being lost and therefore unable to be visualised by microscopy?

II. Might the current suspension method result in slides being prepared from excessively dilute material at ER? If so, then this may potentially lead to a sample being treated as sperm negative, despite enough sperm being present to produce a DNA profile, following Diff Lysis extraction.

In an attempt to answer these questions, the aim of the first part of the project was to investigate the performance of current methods. There was a dearth of relevant in-house experimental data comparing the relative sensitivity of sperm microscopy, AP and p30 detection as it relates to DNA profiling.

Initial experimental testing aimed to measure the relative sensitivity for the detection of spermatozoa and quantify any difference in the number of spermatozoa observed on microscopy slides made from cell suspensions during the ER process, compared to results from slides made during Diff Lysis extraction.

Note: Previously obtained in-house data suggested that current AP and p30 methods have a sensitivity of approximately '1/100' dilution for detecting seminal fluid (Project #78 Verification of ABA cards p30 test; Project #136 – to freeze Acid Phosphatase reagent aliquots for use in Acid Phosphatase screening for seminal fluid). A dilution of approximately '1/20' of neat semen is used for preparing in-house extraction positive control samples (25874V8 Preparation of DNA Quantification Standards & In-house

Quality Controls) and these samples typically yield microscopy results of ~1+ to ~2+ sperm heads, with quantification values between ~0.01 ng/µL (according to positive control log) and ~0.07 ng/µL (average positive control results post processing).

5.2. Part 1; Experiment 1 - Experimental design

Experimental testing for Part 1 of the project was conducted as outlined in the internal document:

"*Project Proposal #181 Investigation into the sensitivity of spermatozoa microscopy; August 2016*"

In summary, mock samples were created following processes outlined within the standard operating procedure (25874V8 Preparation of DNA Quantification Standards & In-house Quality Controls). Liquid semen dilutions of decreasing concentration were created through the addition of Nanopure water, covering the range: '1/ 5'; '1/ 10'; '1/20'; '1/50'; '1/100'; '1/ 200' and '1/ 500'. The semen dilutions were applied to a series of swabs, in the presence of approximately three times as many epithelial cells as are usually added to Diff Lysis positive controls. Buccal cells were used to produce the epithelial cell solution, and the presence of saliva was utilised to allow Phadebas detection sensitivity to be tested in later experiments. Note: the same buccal cell donor was maintained for the duration of the project. The mock samples were intended to emulate the varying proportions of biological material found on typical SAIK swabs. Four replicates of each semen dilution were used, to produce a total of 28 mock samples.

Each mock swab was tested as per in-house procedures for ER examination and microscopy, AP and p30 presumptive screening and Diff Lysis extraction (17189V13 Examination For & Of Spermatozoa; 17186V12 The Acid Phosphatase screening test for seminal stains; 17185V10 Detection of Azoospermic Semen in Casework Samples; 29344V6 DNA IQ™ Extraction using the Maxwell®16).

All samples were tested for AP and p30, regardless of the microscopy results obtained. Following Diff Lysis, microscopy was intended to be conducted on slides prepared from the sperm lysates. Resultant DNA Extracts were to be held pending further investigations.

5.3. Part 1; Experiment 1 - Results

Table 1: Results for Part 1 - Experiment 1

Sample		Examination results			ER Slide			Diff Slide		
Barcode	**Semen Dilution**	**AP**	**AP time (sec)**	**p30**	**Whole sperm**	**Sperm heads**	**Epithelial cells**	**Whole sperm**	**Sperm heads**	**Epithelial cells**
[redacted]	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

AP and p30

- Overall, AP outperformed p30 in terms of detection sensitivity:
  - For AP, the Sensitivity limit (where all four replicates of the same semen dilution achieved a positive result) was at '1/5' semen dilution.
  - For AP, the Detection limit (the most dilute semen concentration at which any positive result was observed) was at '1/20' dilution.
  - For p30, even at '1/5' dilution one of the four replicates gave a negative result, therefore a Sensitivity limit was not established for these results.
  - For p30, the Detection limit was at '1/10' dilution.
- The initial results obtained for AP and p30 testing indicated notably poorer sensitivity than expected, with even relatively concentrated mock samples giving negative results. These findings were inconsistent with internal p30 validation studies.
- Experimental testing was halted before the Microscopy and Diff Lysis extraction of these samples was completed.

5.4. Part 1; Experiment 1 - Discussion

One possible explanation for the reduced sensitivity observed for AP and p30 testing, was that the semen sample used to create the swabs may have been compromised through sub-optimal storage conditions. Excessive dilution was also considered as a potential contributing factor. The addition of liquid from the epithelial cell solution had not been accounted for when calculating the original dilution factor for the mock samples. Due to these possible issues and the poor results obtained, it was decided that Experiment 1 should be repeated and renamed as Experiment 2.

5.5. Part 1; Experiment 2 - Experimental design

Testing was repeated as per Experiment 1 and the internal document:

"*Project Proposal #181 Investigation into the sensitivity of spermatozoa microscopy; August 2016*"

In summary, another set of mock samples were prepared using a fresh stock of semen from the same donor, following processes outlined within the standard operating procedure (25874V9 Preparation of DNA Quantification Standards & In-house Quality Controls). This time, liquid semen dilutions covering the range: '1/50'; '1/100'; '1/ 200' and '1/ 500' were produced and applied to a series of swabs, to which approximately three times as many epithelial cells as usual for Diff Lysis positive controls had been added. When preparing these mock swabs the epithelial (buccal) cell solution was added to each swab first and dried at 35°C (rather than at 56°C). The required volume of diluted liquid semen was then added to the dried swab and again dried at 35°C. This was intended to minimise any dilution caused by the epithelial cell solution. Four replicates of each semen dilution were prepared, to produce a total of 16 mock samples.

Each mock swab was tested as per in-house procedures for ER examination and microscopy, AP and p30 presumptive screening and Diff Lysis extraction (17189V13 Examination For & Of Spermatozoa; 17186V12 The Acid Phosphatase screening test for seminal stains; 17185V10 Detection of Azoospermic Semen in Casework Samples; 29344V6 DNA IQ™ Extraction using the Maxwell®16).

Each sample was tested for AP and p30, regardless of the ER microscopy result obtained. Following Diff Lysis, microscopy was conducted on slides prepared from the sperm lysates. Resultant DNA Extracts were then held pending further investigation.

Note that to calculate the ER microscopy tallies for this and all subsequent experiments in this project (often referred to as the 'Current Method') the 'whole sperm head' and 'sperm head' scores were combined together.

5.6. Part 1; Experiment 2 - Results

Table 2: Results for Part 1 - Experiment 2

Sample		Examination results			ER Slide			Diff Slide		
Barcode	Semen Dilution	AP	AP time (sec)	p30	Whole sperm	Sperm heads	Epithelial cells	Whole sperm	Sperm heads	Epithelial cells
[redacted]	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

Microscopy

- In general, fewer spermatozoa were observed on the ER slides than on the associated Diff Lysis slides, however this difference was relatively small and not unexpected given the concentration of sample volume that occurs during the Diff Lysis process.
- Epithelial cells were observed on all ER slides, mostly in abundant numbers. Epithelial cells were not present on any of the Diff Lysis slides, which was consistent with expected results, due to the separation of cell types that occurs during the Diff Lysis process prior to microscopy.
- As expected, experimental data showed the number of spermatozoa observed on both the ER and Diff Lysis microscopy slides decreased as the semen dilution concentration decreased, in an approximately linear manner.
- For all microscopy slides, the number of spermatozoa observed was relatively consistent across replicates of the same semen concentration.
- There were four results where spermatozoa were observed on the Diff Lysis slide, however no sperm were seen on the ER slide. These occurred at lower concentrations of semen (higher dilutions) and in each case relatively few

spermatozoa were observed on the Diff Lysis slides ('very hard' or 'hard' to find).

AP and p30

- The results obtained for the detection of AP and p30 showed a general improvement in sensitivity, compared to the preliminary results obtained from Experiment 1 and they were broadly in line with expectations from previous internal validation studies.
- Overall, AP outperformed p30 in terms of detection sensitivity:
    - AP Sensitivity limit was '1/50'
    - AP Detection limit was '1/100'
    - p30 Sensitivity limit was '1/10'
    - p30 Detection limit was '1/100'

5.7. Part 1 – Discussion

The original aim of this project was to investigate the performance of the current Evidence Recovery microscopy method. This investigation was initiated after several casework examples were highlighted where spermatozoa were observed in low numbers (or not at all) on the ER slide yet were subsequently observed in relatively large numbers on the associated Diff Lysis slide.

Part 1 of this project attempted to answer two questions:

I. Are spermatozoa potentially being 'lost' during the slide staining procedure?

- Given that epithelial cells were observed on all ER slides prepared from the mock samples and spermatozoa are unlikely to be preferentially 'lost' to a greater degree than other cell types, there was no indication from these results that spermatozoa are getting 'lost' during ER microscopy.

II. Does the current suspension method cause overly diluted samples, thereby affecting the ability to detect spermatozoa on the ER slides?

- Although in general there were more spermatozoa observed on the Diff Lysis slides compared to the ER slides, including four results where spermatozoa were observed only on the Diff Lysis slides, the differences in sperm numbers were relatively small, and not unexpected given that the sample is concentrated during the Diff Lysis extraction process. Large differences between the microscopy results for ER and Diff slides were not observed in the experimental data. Possible explanations for this may include:

- The relatively small sample set;
- The conditions under which the differences were observed in casework not being replicated in this experiment;

- The experiment not being performed as a blind trial;
- Experimental mock samples not being truly representative of casework samples

In conclusion, the data obtained from Part 1 was unable to provide a satisfactory answer to question II.

Following the review of the experimental data, the following two options were considered:

1. Design further experiments to investigate whether there are possible areas to improve on the current ER method.
2. Cease investigating the potential cause of the issue and instead develop and test a novel evidence recovery method.

As part of the decision-making process the following points were noted:

- If an issue with the current method was conclusively identified, then a replacement ER method would be required;
- If no significant issues with the current method can be identified, then the observations in some casework samples with marked differences in 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 worthy of investigation;
- No trends in current anecdotal evidence have been observed to suggest that any particular sample type is preferentially associated with the discrepant ER slide / Diff slide results observed. As the problematic samples represent less than 10% of samples where no spermatozoa are observed from an ER slide, a very large number of test conditions / samples may need to be investigated in order to effectively replicate the observed results experimentally.

It was concluded that it was not feasible to conduct further investigations into the current ER sampling and microscopy methods at this time. Instead it was decided that the next part of the project should focus on developing an improved Evidence Recovery process for semen recovery and detection from Sexual Assault Investigation Kit swabs.

6. Part 2

6.1. Part 2 - Purpose and Scope

The aim of Part 2 of the project was to try and improve the sensitivity of ER microscopy, without adversely affecting the number of spermatozoa available for DNA extraction. Testing was performed to see if spermatozoa were being retained on swabs during ER processing and if so, whether adaptations to the current ER method could improve this aspect of the process. If swab heads are submitted for extraction, then the retention of spermatozoa on those heads may be expected to reduce the quantity of

DNA available from Diff Lysis extraction and, depending on the extraction efficiency, this may affect the success of subsequent DNA profiling.

6.2. Part 2 - Experimental design

An alternative ER process for microscope slide preparation was proposed and investigated.

Experimental testing for Part 2 was conducted as outlined in the internal document:

"*Project Proposal #181 Investigation into the sensitivity of spermatozoa microscopy – Part 2; April 2017*"

In summary, mock samples were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). Liquid semen dilutions of '1/50 and '1/100' concentration were produced and applied to swabs, following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls. The swabs were dried on a hotblock set to 35°C, rather than the 56°C stipulated in the standard procedure. Six replicates of each semen dilution were used, to produce a total of 12 mock samples.

Each mock swab was tested as per in-house procedures for ER examination and microscopy (17189V14 Examination For & Of Spermatozoa), with the following adaptations (No AP or p30 testing was performed):

1. Swab transferred to a spin basket and centrifuged for 2 minutes at maximum speed;
2. Supernatant and spin basket swab were transferred to new (separate) tubes, leaving the cell pellet and a small amount of supernatant to be resuspended. A second microscope slide was prepared from the resuspended pellet and read with the initial slide.
3. Cell pellet and swab from spin basket were submitted for DNA profiling as separate samples.

Each sample (now totalling 24) underwent Diff Lysis extraction as per in-house procedures (34044V2 DNA IQ™ Extraction using the Maxwell®16). Microscope slides were prepared and read for each sperm lysate. The resultant DNA Extracts were held pending further investigations. The results obtained from the ER and Diff Lysis microscopy were compared.

6.3. Part 2 - Results

Table 3: Results for Part 2

Sample		ER Slide			ER slide post spin			Diff Slide (spin basket)		Diff Slide (pellet)	
Barcode	**Semen dilution**	**Whole sperm**	**Sperm heads**	**Epithelial cells**	**Whole sperm**	**Sperm heads**	**Epithelial cells**	**Sperm heads**	**Epithelial cells**	**Sperm heads**	**Epithelial cells**
[redacted]	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
	1 in 100 #3	<1+	1+	3+	<1+	1+	4+	1+	0	1+	0
	1 in 100 #4	1+	1+	4+	<1+	1+	3+	1+	0	1+	0
	1 in 100 #5	1+	1+	4+	<1+	1+	3+	1+	0	1+	0
	1 in 100 #6	1+	1+	4+	<1+	1+	3+	1+	0	1+	0

Microscopy

- At least 1+ sperm heads were detected on all slides from Diff Lysis microscopy. These results indicate significant numbers of spermatozoa are still being retained in the swab head after initial ER processing.
- In general, the number of sperm heads detected in the 'ER post spin' slide (i.e. from the cell pellet) was fewer than the number detected in the 'ER' slide and approximately the same as that detected in either Diff Lysis slide. These results suggest that spermatozoa are also being retained during the alternative process.
- There was no instance of any Diff Lysis slides showing a higher number of spermatozoa than the initial ER slide. This may be due to the number of sperm heads available for detection tending to decrease as each slide is produced.
- Epithelial cells were observed on all ER slides in abundant numbers and were not present on any Diff Lysis slides. This was not unexpected, due to the separation of cell types occurring during the Diff Lysis process prior to microscopy.

6.4. Part 2 – Discussion

The aim of this experiment was to determine whether the sensitivity of Evidence Recovery microscopy could be improved and to determine whether spermatozoa are being retained in swabs during the current ER method.

The results showed that spermatozoa are still being retained on swabs during the current method and are also retained during the proposed adapted method. This suggests that using a spin basket to create a pellet at ER microscopy does not appear to be advantageous.

Following a review of the experimental data, two options were considered for the next stage of the Project:

1. Attempt to improve the ER process further with a view to releasing more spermatozoa from the swab head.
2. Submit sexual assault swabs straight for Diff Lysis extraction and only proceed to DNA profiling if spermatozoa are detected on the Diff Lysis slide.

As part of the decision-making process, the following considerations were made for each option:

Option 1

- The process downstream from ER for the Analytical team may become more efficient as the requirement to prepare a Diff Lysis slide may be eliminated; however, ER processing may become slower and more laborious;
- Would necessitate further method development;
- The capacity for AP / p30 presumptive testing with current methodology could be maintained;
- This option may be more cost effective than performing a Diff Lysis extraction on all swabs.

Option 2

- Discontinuing slide preparation at ER may decrease the loss of spermatozoa from samples during screening, potentially increasing the likelihood of obtaining a DNA profile if only minimal sperm are present;
- ER processing would become more efficient, at the expense of an increased workload for the Analytical Team as all samples would follow Diff Lysis pathway;
- Y-quant could potentially be used to direct workflow, with further method development;
- Could be implemented immediately, although FR / workflow development would be required;
- Would lose capacity for AP / p30 presumptive testing, which would be a significant disadvantage.

Outcome

It was decided that a modified version of Option 2 should be developed, with the objective of trying to improve spermatozoa preservation whilst still maintaining the capacity for AP / p30 presumptive testing.

7. Part 3

7.1. Part 3 - Purpose and Scope

This part of the project explored the viability of submitting all swabs that require semen testing directly for Diff Lysis extraction, without first performing microscopy at ER. A modification of the examination process for SAIK swabs was tested, with the aim of preserving spermatozoa whilst still retaining the ability to perform presumptive screening (AP, p30 and Phadebas).

7.2. Part 3 - Experimental design

Experimental testing for Part 3 of the project was conducted as outlined in the internal document:

Project Proposal #181 – Investigation into the sensitivity of spermatozoa microscopy – Part 3; May 2018

In summary, mock samples were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). Liquid semen dilutions of '1/100', '1/200' and '1/500' concentration were produced and applied to swabs, following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls. The swabs were dried on a hot block set to 35°C. Four replicates of each dilution were used, to produce a total of 12 mock samples.

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 informative data about the sensitivity thresholds of AP, p30 and sperm microscopy.

<u>ER Processing</u>

Half of the mock samples were processed as per in-house procedures for ER microscopy, AP and p30 (17189V14 Examination For & Of Spermatozoa; 17186V12 The Acid Phosphatase screening test for seminal stains; 17185V11 Detection of Azoospermic Semen in Casework Samples). These mock samples were described as following the "current method".

The remaining six mock samples underwent the adapted method detailed below, described as the "proposed method":

1. Swab heads sampled into a 1.5 mL tube (Note: semen in-tubes are received in 2.0 mL tubes);

2. 650 µL Nanopure water added to the swab head;
3. Vortex mix, incubate for 30 mins at room temperature (RT) (as per current "retain supernatant" method);
4. Vortex mix, centrifuge for 2 mins at maximum speed (Note: swab remains in the tube, no need to transfer cell pellet).
5. Transfer 150 µL of supernatant into a new 1.5 mL tube (new barcode, subsample type "SUPNAT" – to allow for possible Phadebas testing); transfer an additional 300 µL into a different 1.5 mL tube (new barcode, subsample type "MISC" – for any potential AP and/or p30 testing);
   a. MISC & SUPNAT to be stored frozen for 1 week prior to processing, to replicate maximum routine processing timeframes.
6. Samples submitted for routine Diff Lysis DNA extraction.

DNA Extraction

- Both sets of mock samples underwent routine processing for Diff Lysis Extraction (34044V2 DNA IQ™ Extraction using the Maxwell®16).
- Mock samples following 'current' ER method underwent the Diff Lysis (Retain Supernatant) method.
- Mock samples following the 'proposed' method underwent the Diff Lysis (No Retain Supernatant) method.
- Spermatozoa fractions were held post-extraction, pending Diff Lysis microscopy and AP / p30 testing (if required).

Microscopy and Presumptive testing

- Slides from ER and Diff Lysis microscopy were read, and the results were compared.
- AP and p30 testing were conducted upon all mock samples following either the proposed method (on the retained "MISC" supernatants) or the current method.
- Phadebas testing was performed on all mock samples, including the DLYS retain supernatants for the current method, and the retained "SUPNAT" for the proposed method.

7.3. Part 3 - Results

Table 4: Results for Part 3 - Current method

Sample		ER Slide			ER Presumptive tests			Diff slide	Diff Supernatant
Barcode	Semen dilution	Whole sperm	Sperm heads	Epithelial cells	AP	time (s)	p30	Sperm heads	Phadebas
[redacted]	1/100	1+	<1+	3+	pos	58	neg	1+	pos
	1/100	<1+	<1+	3+	pos	60	neg	1+	pos
	1/200	<1+	0	2+	pos	87	neg	<1+	pos
	1/200	<1+	<1+	3+	pos	104	neg	<1+	pos
	1/500	<1+	0	3+	neg		neg	<1+	pos
	1/500	<1+	<1+	2+	neg		neg	<1+	pos

Table 5: Results for Part 3 - Proposed method

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	1/100	2+	pos	60	neg	pos
	1/100	2+	pos	65	pos	pos
	1/200	1+	neg		neg	pos
	1/200	2+	neg		neg	pos
	1/500	1+	neg		neg	pos
	1/500	1+	neg		neg	pos

Microscopy

- Spermatozoa were microscopically detected on all mock samples for both the current and proposed methods.
- The microscopy results obtained from the proposed method had more sperm heads observed than either the ER slides or the Diff Lysis slides from the current method.
- The microscopy results obtained from the ER slides and the Diff Lysis slides for the current method were relatively similar to each other.

AP

- The current method outperformed the proposed method for the detection sensitivity of AP.
- Current method;
    - AP Sensitivity limit was '1/200'
    - AP Detection limit was '1/200'
- Proposed method;
    - AP Sensitivity limit was '1/100'
    - AP Detection limit was '1/100'

p30

- The proposed method slightly outperformed the current method for the detection sensitivity of p30 and overall AP outperformed p30:
- Current method;
    - All dilutions tested negative for p30
- Proposed method;
    - p30 Detection limit was '1/100' (one single replicate only)

Phadebas

- All samples gave positive results for Phadebas, for each method type.

7.4. Part 3 – Discussion

The aim of this experiment was to compare microscopy, AP, p30 and Phadebas results for samples processed using the 'current method' (including both ER and Diff Lysis slides) against those produced from an adapted 'proposed method'. It should be noted that this data was produced from quite a limited experimental sample set.

For microscopy, the proposed method demonstrated superior sensitivity. Across all presumptive screening tests, the two methods were generally comparable, although the current method outperformed the proposed method in terms of AP sensitivity and the proposed method outperformed the current method in terms of p30 detection. Overall AP detection was more sensitive than p30 in these results.

As previously noted, in-house data suggests that current AP and p30 methods have a sensitivity of ~'1/100' dilution when detecting seminal fluid, therefore the dilutions used for these mock samples are approaching the expected Limit of Detection (LOD) for these presumptive tests. Given this and considering the small sample set, the AP testing performance for the proposed method was considered a relatively minor concern at this stage, although worthy of further investigation.

Based on the promising results obtained for the proposed method, the next stages of the project were intended to further develop and validate the new process by exploring an expanded range of experimental variables in order to better replicate live casework. This testing was to involve several different elements:

- Increasing the number of different semen donors.
- Extending the range of semen dilutions to try and establish effective detection sensitivities for the different test methods.
- Expanding the range of substrates, to cover the main types commonly submitted for routine casework (swabs and fabrics)
- Expand the range of fabric sampling techniques, to include all methods regularly used during routine casework.
- Explore whether reducing the volume of water added to the swab during the proposed method has any effect (Note: the minimum volume of water required is constrained by presumptive testing requirements and substrate type).
- Test whether variations in incubation time and temperature conditions have any effect on the proposed method, with a view to optimising AP / p30 sensitivity, whilst limiting any associated negative effects on Microscopy or Phadebas results.

8. Part 4

8.1. Part 4 - Purpose and Scope

This part of the project was designed to see whether variations in incubation time and temperature conditions affected the results obtained for the 'proposed method' (as described in Part 3). The aim here was optimisation of AP / p30 test sensitivity, whilst minimising any associated negative effects on microscopy or Phadebas results. 'Optimal' incubation conditions could then be applied to further experiments when testing other variables.

8.2. Part 4 - Experimental design

Experimental testing for Part 4 of the project was conducted as outlined in the internal document:

*Project Proposal #181 – Investigation into the sensitivity of spermatozoa microscopy – Part 4; March 2019; (**Section 6**)*

In summary, mock samples were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). Liquid semen dilutions of '1/100', '1/200' and '1/500' concentration were produced and applied to swabs, following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls. The swabs were dried on a hot block set to 35°C. Ten replicates of each dilution were used, to produce a total of 30 mock samples.

Note that there was a change to the semen donor for this experiment, so that although some conditions were repeated from Part 3, a degree of variation between each set of results would not be unexpected.

Mock swabs were processed either as per in-house procedures for ER examination and microscopy (17189V15 Examination For & Of Spermatozoa) or using the proposed method (described in Part 3), incorporating variation in incubation conditions (duration and temperature) as shown in Table 6. Note: 'incubation' referred to relates to the sample processing stage, rather than to microscopy slide preparation.

Table 6: Variation in Incubation Conditions for Part 4

Semen dilution	Current method	Proposed method			
		15 min @ RT°C	**15 min @ 30°C**	**30 min @ RT°C**	**30 min @ 30°C**
1/100	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/200	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/500	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs

AP and p30 presumptive screening and a Diff Lysis extraction process were conducted on all mock swabs as per in-house procedures (17186V12 The Acid Phosphatase screening test for seminal stains; 17185V10 Detection of Azoospermic Semen in Casework Samples; 29344V6 DNA IQ™ Extraction using the Maxwell®16).

As before, the results from Microscopy, AP, p30 and Phadebas testing were collated and compared for all mock samples.

8.3. Part 4 - Results

Table 7: Results for Part 4 - current method

Sample		ER Slide			ER Presumptive tests			Diff slide	Diff Supernatant
Barcode	Semen dilution	Whole sperm	Sperm heads	Epithelial cells	AP	time (s)	p30	Sperm heads	Phadebas
[redacted]	1/100	0	<1+	1+	pos	60	neg	2+	pos
	1/100	<1+	1+	2+	pos	60	pos	1+	pos
	1/200	0	<1+	1+	neg		neg	1+	pos
	1/200	0	<1+	1+	neg		neg	1+	pos
	1/500	<1+	0	1+	neg		neg	<1+	pos
	1/500	0	<1+	1+	neg		neg	<1+	pos

Table 8: Results for Part 4 - proposed method (15 min @ RT°C)

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	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

Table 9: Results for Part 4 - proposed method (30 min @ RT°C)

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	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

Table 10: Results for Part 4 - proposed method (15 min @ 30°C)

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	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

Table 11: Results for Part 4 - proposed method (30 min @ 30°C)

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	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	1+	neg		neg	pos
	1/500	<1+	neg		neg	pos

Microscopy

- Detection sensitivity for sperm microscopy was superior to either AP or p30 testing. Across all results, for both current and proposed methods, only one replicate did not achieve a positive detection for sperm (Proposed Method; '1/500'; incubated for 15 min @ RT).
- Proposed method sperm microscopy produced superior detection sensitivity to current method ER microscopy and comparable detection to Diff Lysis slides.

AP

- AP results were generally poor, and the current method outperformed the proposed method for the detection sensitivity of AP
- Current method;
    - AP Sensitivity limit was '1/100'
    - AP Detection limit was '1/100'
- Proposed method;
    - All dilutions tested negative for AP

p30

- Overall p30 testing was more sensitive than AP testing, particularly for the proposed method.

- The proposed method outperformed the current method for p30 detection sensitivity:
- Current method;
    - p30 Detection limit was '1/100' (one replicate only)
- Proposed method;
    - Best p30 Sensitivity limit was '1/200' (30 min @ RT)
    - Best p30 Detection limit was at '1/200' (30 min @ RT and 15 min @ ~30°C)

Phadebas

- All samples gave positive results for Phadebas, for both method types.

8.4. Part 4 – Discussion

The aim of this part of the Project was to compare microscopy, AP, p30 and Phadebas results for samples processed using the current and proposed methods under different incubation conditions. Whilst noting the limited sample set, both methods generally produced comparable outcomes, with results from the 'proposed' method producing equivalent or improved sensitivity to the 'current' ER method at each semen dilution tested.

Two sets of incubation conditions were considered as optimal based upon these findings ('30 min at RT' and '15 min at 30°C'). These conditions resulted in improved sensitivity for p30 testing and slightly higher sperm counts at microscopy.

AP results from the proposed method were unsatisfactory, with no positive detections recorded for any mock samples. The next experiment explored AP test sensitivity further.

9. Part 5

9.1. Part 5 - Purpose and Scope

As no positive AP results had been obtained for the proposed method in the previous experiment, the sensitivity of the method required further investigation. Two questions were raised:

I. Is there a dilution effect impacting upon the AP results?

II. Could the freezing of sample supernatants prior to conducting the AP test have a deleterious effect on detection sensitivity?

The aim of the next experiment was to investigate the performance of AP and attempt to answer these questions. To account for natural variability in the constituent

components of semen (AP / p30 activity and spermatozoa count), the number of semen donors was increased.

9.2. Part 5 - Experimental design

Experimental testing for Part 5 of the project was conducted as outlined in the internal document:

*Project Proposal #181 – Investigation into the sensitivity of spermatozoa microscopy – Part 4 – additional testing; June 2019; (**Section 5.2**)*

In summary, mock samples were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). For this experiment liquid semen samples collected from five donors were diluted to cover the range: '1/50', '1/100', '1/200', '1/500' concentration. The semen donors included the individuals used for the experiments featured in Parts 1 – 3 ('Donor 4'); Part 4 ('Donor 5'); and three additional semen donors ('Volunteers 1 – 3'). The semen dilutions were applied to swabs, following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls. The swabs were dried on a hot block set to 35°C.

Mock samples were processed following either 'current' ER procedures for ER examination and microscopy (17189V15 Examination For & Of Spermatozoa) or the 'proposed' method as described in Part 3. Two sets of 'optimal' incubation conditions ('30 min @ RT' and '15 min @ 30°C') from Part 4 were utilised for the proposed method. Two replicate mock swabs at each semen dilution level, from each semen donor were used, as shown in Table 12.

Table 12: Swab Incubation Conditions – for each of 5 semen donors

Semen dilution	Current method	Proposed method	
		15 min @ 30°C	**30 min @ RT°C**
1/50	2 swabs	2 swabs	2 swabs
1/100	2 swabs	2 swabs	2 swabs
1/200	2 swabs	2 swabs	2 swabs
1/500	2 swabs	2 swabs	2 swabs

In total, 120 mock swabs were prepared for this experiment:

- 40 from the 'current method'
- 40 from the proposed method (at '30 min @ RT')
- 40 from the proposed method (at '15 min @ 30°C')

AP Testing

AP screening was conducted as per in-house procedures (17186V13 The Acid Phosphatase screening test for seminal stains).

AP tests were performed upon each mock sample which underwent the 'current method'. For the 'proposed method', AP testing was performed on a subsample of each supernatant, before freezing any remaining supernatant and retesting it again seven days later. The remaining supernatant was then refrozen. After another week, the remaining supernatant was retested a third time using a new batch of AP reagent solution, prepared with a fresh batch of the active ingredient, sodium α-naphthyl phosphate.

Mock samples processed using the current method were AP tested once. The supernatant from each swab following the 'proposed method' was AP tested three times (see Table 3). In total, 280 AP tests were conducted during this experiment.

Table 13: AP testing (two replicates of each of 4 dilutions, for each of 5 semen donors)

Current method	Proposed method	
	30 min @ RT	**15 min @ 30°C**
AP	AP	AP
	AP after 7 days	AP after 7 days
	Fresh AP after 14 days	Fresh AP after 14 days

9.3. Part 5 - Results

Table 14: AP testing, Part 5 - Current method

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	**Time (s)**	**AP**	**Time (s)**	**AP**	**Time (s)**	**AP**	**Time (s)**	**AP**	**Time (s)**
1/50	neg		pos	60	neg		pos	110	pos	80
1/50	neg		pos	40	pos	100	pos	110	neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 15: AP testing, Part 5 - Proposed method (initial AP testing, 30 min @ RT)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		neg		neg		neg		neg	
1/50	neg		pos	80	neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 16: AP testing, Part 5 - Proposed method (initial AP testing, 15 min @ 30°C)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		pos	65	neg		neg		neg	
1/50	neg		pos	60	neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 17: AP testing, Part 5 - Proposed method (AP testing 1 week, 30 min @ RT)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		neg		neg		neg		pos	100
1/50	neg		pos	70	neg		neg		pos	120
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 18: AP testing, Part 5 - Proposed method (AP testing 1 week, 15 min @ 30°C)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		neg		neg		neg		neg	
1/50	neg		pos	65	neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 19: AP testing, Part 5 - Proposed method (new AP reagent 14 days, 30 min @ RT)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		neg		neg		neg		neg	
1/50	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

Table 20: AP testing, Part 5 - Proposed method (new AP reagent 14 days, 15 min @ 30°C)

Semen dilution	Donor 4		Donor 5		Volunteer 1		Volunteer 2		Volunteer 3	
	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)	AP	Time (s)
1/50	neg		neg		neg		neg		neg	
1/50	neg		pos	120	neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/100	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/200	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	
1/500	neg		neg		neg		neg		neg	

AP

- AP results were generally poor, with positive detection achieved with relatively few results. There were no positive results for any semen dilution less concentrated than '1/50'.
- Variation in positive AP detection was evident between semen originating from different donors, ranging between:
    - Donor 4 - Detected AP in 0 out of 56 tests (0 out of 14 of the '1/50' dilutions)
    - Donor 5 - Detected AP in 8 out of 56 tests (8 out of 14 of the '1/50' dilutions)
- The current method slightly outperformed the proposed method for the detection sensitivity of AP.
- Current method;
    - Detected AP in 6 out of 50 tests (6 out of 10 of the '1/50' dilutions).
    - Best AP Sensitivity limit was '1/50' (Donor 5 and Volunteer 2)
    - Best AP Detection limit was '1/50'
- Proposed method;

- Detected AP in 8 out of 240 tests (8 out of 60 of the '1/50' dilutions). Note: 240 tests comprised 80 supernatants each tested three times.
- Best AP Sensitivity limit was '1/50' (Donor 5; initial AP and Volunteer 3; AP after 7 days)
- Best AP Detection limit was '1/50'

- For the Proposed method, there was no apparent difference between results obtained from either set of incubation conditions:
  - '30 min @ RT' - Detected AP in 4 out of 120 tests (4 out of 30 of the '1/50' dilutions)
  - '15 min @ 30°C' - Detected AP in 4 out of 120 tests (4 out of 30 of the '1/50' dilutions)
- For the proposed method results, there was no apparent difference in AP detection between initial testing of the liquid supernatant and retesting after freezing for 7 days. After 14 days, with a fresh batch of AP reagent there was some reduction in the detection frequency.
  - Initial testing - Detected AP in 4 out of 80 tests (4 out of 20 of the '1/50' dilutions)
  - After 7 days frozen - Detected AP in 4 out of 80 tests (4 out of 20 of the '1/50' dilutions)
  - After 14 days frozen (+ fresh reagent) - Detected AP in 1 out of 80 tests (1 out of 20 of the '1/50' dilutions)

9.4. Part 5 – Discussion

After this experiment the results obtained for AP detection sensitivity remained unsatisfactory, with relatively few tests indicating a positive reaction under a range of test conditions. Semen dilutions of '1/50' appeared to be close to the Limit of Detection for AP, but positive detection was very inconsistent even at this relatively concentrated dilution level. Despite the generally poor results, the current method slightly outperformed the proposed method for the detection sensitivity of AP, which is not ideal for development of this method.

Across the different variables explored in this experiment, the most notable difference in the results was associated with changing the semen donor. This finding supports the expectation that natural variation occurs within the constituent component elements of human semen, and that establishing generally applicable sensitivity limits for a particular component (such as AP) is very challenging.

Most other variables tested using the proposed method, appeared to only minimally impact results. There was no apparent advantage seen when using a particular set of incubation conditions, for testing supernatants immediately as opposed to being frozen for 7 days, or for using fresh AP solution. Variations in these conditions may be expected to occur during typical casework processing.

Note: Although a decrease in AP detection was seen after 14 days, the frequencies for positive detection were so low overall that this difference may not be significant.

10. Part 6

10.1. Part 6 - Purpose and Scope

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.

The aim of the next experiment was to see whether the 'proposed' method could be improved, by reducing the volume of water added to the swab head at ER, in order to increase the sensitivity of subsequent presumptive tests. The volume of additive water is only able to be reduced to the extent that the substrate is still adequately moistened, and sufficient volume remains to be able to perform any required presumptive testing.

This experiment also incorporated further variations to the incubation conditions, including the application of physical agitation through the use of a 'thermomixer' as opposed to a stationary 'heatblock', in order to see if this had any effect upon the detection sensitivity of the standard presumptive screening tests for either the 'current' or 'proposed' methods.

10.2. Part 6 - Experimental design

Experimental testing for Part 6 of the project was conducted as outlined in the internal document:

*Project Proposal #181 – Investigation into the sensitivity of spermatozoa microscopy – Part 4 – additional testing; June 2019; (**Sections 5.2 (Additional Testing) and 6.2**)*

In summary, mock samples were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). For this experiment, the semen sample collected from Donor 5 was used as this had given the best results for AP detection sensitivity in Part 5. Neat semen dilutions covering the range '1/20', '1/50', 1/100', '1/200 and '1/500' were prepared and applied to swabs following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls. The swabs were dried on a 35°C hot block.

Mock samples were processed following either current ER procedures or the proposed method described in Part 3, incorporating some changes, as described below. For both the current and proposed methods, variations in incubation conditions (duration, temperature and agitation) were individually tested, as shown in Table 3. For each set

of experimental conditions, two duplicate swabs at each dilution level were used, producing a total of 70 mock samples (see Table 21).

Table 21: Variation in Incubation Conditions

Semen dilution	Current ER method			Proposed ER method			
	Standard process	**30 min @ RT°C**	**30 min @ 30°C**	**15 min @ RT°C**	**15 min @ 30°C**	**30 min @ RT°C**	**30 min @ 30°C**
1/20	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/50	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/100	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/200	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs
1/500	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs	2 swabs

Note: For the incubation step, one swab from each duplicate pair was agitated on a thermomixer, set to shake at 1400 rpm at the required temperature (22°C for samples at 'room temperature'). The remaining swabs were incubated using a standard hotblock, without applying agitation. Mock swabs following the current ER method were neither incubated nor agitated.

The following changes were made to the 'proposed' method (as described in Part 3):

- 400 µL of Nanopure water was added to each swab head, instead of 650 µL;
- Only 200 µL supernatant was removed for AP / p30 testing, instead of 300 µL;
- No supernatant was removed for Phadebas testing; instead samples were submitted for "SUPNAT" Diff Lysis 'retain supernatant' DNA extraction, to allow for Phadebas testing;
- Supernatants were frozen, stored and then tested after one week, to emulate typical casework practice;
- Additional testing of the current method was conducted, incorporating an incubation period before the preparation of the ER microscopy slide. This method proceeded as per current in-house procedures (33800V3 Examination of Items (Forensic Register); 17189V14 Examination for & of Spermatozoa) with the following changes:
    - Before slide creation, the sample was incubated for 30 minutes at either room temperature or ~30°C (ensuring the samples are vortex mixed before incubation);
    - Vortex mix tube after incubation and before creation of microscopy slide;

10.3. Part 6 - Results

Table 22: Current method, routine conditions

Sample		ER Slide			ER Presumptive tests			Diff slide	Diff Supernatant
Barcode	Semen dilution	Whole sperm	Sperm heads	Epithelial cells	AP	time (s)	p30	Sperm heads	Phadebas
[redacted]	1/20	0	<1+	<1+	pos	20	pos	3+	pos
	1/20	0	<1+	<1+	pos	30	pos	3+	pos
	1/50	0	<1+	0	pos	60	neg	2+	pos
	1/50	0	<1+	1+	pos	60	pos	2+	pos
	1/100	0	<1+	1+	pos	70	neg	1+	pos
	1/100	<1+	<1+	0	pos	70	neg	1+	pos
	1/200	<1+	<1+	1+	pos	90	pos	2+	pos
	1/200	<1+	1+	1+	neg		neg	1+	pos
	1/500	0	0	<1+	neg		neg	<1+	pos
	1/500	0	0	1+	neg		neg	<1+	pos

Table 23: Current method, incubation for 30 min @ RT

Sample		ER Slide			ER Presumptive tests			Diff slide	Diff Supernatant
Barcode	Semen dilution	Whole sperm	Sperm heads	Epithelial cells	AP	time (s)	p30	Sperm heads	Phadebas
[redacted]	1/20	<1+	<1+	1+	pos	30	pos	3+	pos
	1/20	0	<1+	<1+	pos	25	pos	4+	pos
	1/50	0	<1+	<1+	pos	60	neg	1+	pos
	1/50	<1+	1+	1+	pos	60	pos	1+	pos
	1/100	0	<1+	<1+	pos	90	neg	1+	pos
	1/100	<1+	<1+	1+	neg		neg	1+	pos
	1/200	0	0	<1+	neg		neg	1+	pos
	1/200	<1+	<1+	<1+	neg		neg	1+	pos
	1/500	0	<1+	<1+	neg		neg	<1+	pos
	1/500	0	0	<1+	neg		neg	<1+	pos

Note: samples marked with * were agitated in a thermomixer during incubation

Table 24: Current method, incubation for 30 min @ 30°C

Sample		ER Slide			ER Presumptive tests			Diff slide	Diff Supernatant
Barcode	Semen dilution	Whole sperm	Sperm heads	Epithelial cells	AP	time (s)	p30	Sperm heads	Phadebas
[redacted]	1/20	<1+	1+	1+	pos	30	pos	2+	pos
	1/20	<1+	1+	1+	pos	50	pos	3+	pos
	1/50	0	0	<1+	pos	60	neg	2+	pos
	1/50	0	0	0	pos	110	neg	1+	pos
	1/100	0	<1+	<1+	pos	95	neg	1+	pos
	1/100	0	<1+	0	pos	120	neg	1+	pos
	1/200	0	<1+	0	pos	120	neg	1+	pos
	1/200	0	<1+	1+	neg		neg	1+	pos
	1/500	0	0	0	neg		neg	<1+	pos
	1/500	0	0	0	neg		neg	<1+	pos

Note: samples marked with * were agitated in a thermomixer during incubation

Table 25: Proposed method, incubation 15 min @ RT

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	1/20	3+	pos	50	pos	pos
	1/20	4+	pos	50	pos	pos
	1/50	1+	pos	120	pos	pos
	1/50	2+	pos	90	pos	pos
	1/100	2+	neg		pos	pos
	1/100	1+	neg		neg	pos
	1/200	2+	neg		pos	pos
	1/200	1+	neg		neg	pos
	1/500	<1+	neg		neg	pos
	1/500	1+	neg		neg	pos

Note: samples marked with * were agitated in a thermomixer during incubation

Table 26: Proposed method, incubation 30 min @ RT

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	1/20	3+	pos	60	pos	pos
	1/20	3+	pos	70	pos	pos
	1/50	2+	pos	90	pos	pos
	1/50	1+	pos	110	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

Note: samples marked with * were agitated in a thermomixer during incubation

Table 27: Proposed method, incubation 15 min @ 30°C

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	1/20	3+	pos	60	pos	pos
	1/20	4+	pos	60	pos	pos
	1/50	2+	pos	110	pos	pos
	1/50	3+	pos	100	pos	pos
	1/100	1+	neg		pos	pos
	1/100	1+	neg		pos	pos
	1/200	1+	neg		neg	pos
	1/200	1+	neg		pos	pos
	1/500	<1+	neg		neg	pos
	1/500	<1+	neg		neg	pos

Note: samples marked with * were agitated in a thermomixer during incubation

Table 28: Proposed method, incubation 30 min @ 30°C

Sample		Diff Slide	Presumptive testing			
Barcode	Semen dilution	Sperm heads	AP	Time (s)	P30	Phadebas
[redacted]	1/20	3+	pos	55	pos	pos
	1/20	4+	pos	70	pos	neg
	1/50	2+	pos	85	pos	pos
	1/50	1+	neg		pos	pos
	1/100	1+	neg		pos	pos
	1/100	1+	neg		neg	neg
	1/200	1+	neg		neg	neg
	1/200	1+	neg		neg	neg
	1/500	<1+	neg		neg	pos
	1/500	<1+	neg		neg	pos

Note: samples marked with * were agitated in a thermomixer during incubation

Microscopy

- Results were generally very good for the 'proposed method', with spermatozoa detected on all slides, even down to the least concentrated semen dilutions ('1/500').
- Results were also good for the Diff Lysis slides; comparable to the equivalent 'proposed method' results, with spermatozoa detected on every slide.
- Both the 'proposed method' and Diff Lysis microscopy results were superior to the 'current method' ER microscopy results for sperm detection sensitivity.

AP

- As in Part 5, the current method outperformed the proposed method for the detection sensitivity of AP:
- Current method;
    - Results obtained were superior to those from equivalent tests in Part 5
    - Detected AP in 19 out of 30 tests.
    - AP Sensitivity ranged between '1/50' and '1/100'
    - AP Detection limit ranged between '1/100' and '1/200'
- Proposed method;
    - Detected AP in 15 out of 40 tests
    - AP Sensitivity limit ranged between '1/20' and '1/50'
    - AP Sensitivity was more consistent than in Part 5 (AP positive detected in 7 out of 8 of the '1/50' dilutions)
    - AP Detection limit was '1/50'

p30

- The proposed method slightly outperformed the current method for the detection sensitivity of p30:
- Current method;
    - p30 Sensitivity Limit was '1/20'
    - p30 Detection Limit was '1/20' to '1/200'
- Proposed method;
    - p30 Sensitivity Limit was '1/50' to '1/100'
    - p30 Detection Limit was '1/100' to '1/200'
- For the proposed method, the results obtained for p30 detection sensitivity outperformed the equivalent results for AP.
- Compared to the detection sensitivity of sperm microscopy, the p30 results were relatively inferior, however, they were still comparable with previous validation studies once initial dilution factors were considered.

Phadebas

- Nearly all mock samples produced positive Phadebas results (for both current and proposed methods).

Incubation

- For the proposed method, there was no single set of incubation conditions which conclusively outperformed all others, however the best results in terms of both sperm count at microscopy and p30 sensitivity detection limits were observed at '15 mins @ 30°C'.
- For the current method, there was no single set of incubation conditions which conclusively outperformed all others, with most detection sensitivities being generally comparable across the data.
- For both p30 and Microscopy there appeared to be a slight advantage to applying agitation during incubation by using the thermomixer, as opposed to the heatblock.
- 5 of 6 negative Phadebas results came from samples incubated on a thermomixer.

10.4. Part 6 - Discussion

The results obtained from this experiment showed AP and p30 detection were not as sensitive as sperm microscopy, across the range of test conditions and for either current or proposed method. It is encouraging that the sperm microscopy for the proposed method (and diff lysis slides) showed excellent detection sensitivity across the range of semen dilutions.

As in Part 5, AP detection was less sensitive for the proposed method than for the current method. Reducing the volume of additive water did not improve the AP detection limit for the proposed method, however it may have contributed to making the sensitivity more consistent, as only one result failed to detect AP at the '1/50' level. The current method results for AP were generally superior to the equivalent results from part 5, so it should be considered that perhaps other aspects of the experimental process or purely random effects may also affect results. It should also be noted that when conducting AP testing, the mock samples are still approximately twice as diluted in the proposed method as they are in the current method.

Variations in incubation conditions (temperature, duration and agitation) were also explored in this part of the project. For the proposed method, the results did not indicate any significant advantage to a particular set of conditions, however '15 mins @ ~30°C' was judged to be the best overall in terms of microscopy and p30 sensitivity. For the current method, there was no obvious difference between results obtained from the various different incubation conditions, therefore this aspect was not considered worthy of further investigation.

For p30 and microscopy there appeared to be a slight advantage to applying agitation during incubation (using a thermomixer as opposed to the standard heatblock), however the majority of the (few) negative Phadebas results came from mock samples incubated on a thermomixer. This aspect was investigated further in the experiment described in Part 7.

11. Part 7

11.1. Part 7 - Purpose and Scope

This part of the project was designed to further expand the testing of the 'proposed' method described in Part 3, to cover alternate substrates, including different types of fabric and swabs. The intention here was to emulate the range of sample substrates commonly encountered during sexual assault casework. Sampling of fabric mock samples was undertaken using three standard techniques commonly used by ER examining scientists: scraping, tape-lifting and excision. For the proposed method to be considered acceptable for use in live casework, equivalent or superior results were required across all tests, when using the proposed method, compared to the current method.

This experiment was conducted in two parts:

- Initial testing was performed on scrapings of mock samples prepared from two representative types of fabric, seeded with epithelial cell solution and diluted semen from the five donors used in Part 5.
- After reviewing the initial data, follow-up testing was performed on tape-lifts and excisions of two different fabric types and another type of swab. For this second lot of testing only semen dilutions from the three donors that gave the best results from Part 5 were used.

11.2. Part 7 - Experimental design

Experimental testing for Part 7 of the project was conducted as outlined in the internal document:

*Project Proposal #181 – Investigation into the sensitivity of spermatozoa microscopy – Part 4 – additional testing; June 2019; (**Section 6.3**)*

In summary, mock samples were created by preparing DNA-free fabric using an in-house derived method outlined within the standard operating procedure (24123V11 Proficiency Testing in DNA Analysis (Appendix A)).

The following fabric types were selected as being representative of substrates commonly submitted for casework examination:

- thin fabric (e.g. cotton blend, commonly found in underwear, lightweight clothing etc.);
- thick fabric (e.g. bath towel type material).

Fabric pieces were washed in a bleach solution before sodium sulphite was added. The fabric was rinsed three times in Nanopure water and allowed to dry. Once dry, an area was marked on each piece of fabric.

Liquid semen samples from the five donors used in Part 5 were diluted to cover the range: '1/20', '1/50', '1/100', '1/200', '1/500'. Semen dilutions were applied to the marked area on each mock fabric sample, following the addition of approximately three times as many epithelial (buccal) cells as are normally added to Diff Lysis positive controls.

Sampling of the marked areas on the fabric pieces proceeded either by scraping, tape-lifting or excision according to standard laboratory procedures.

The swabs used at Forensic DNA Analysis, including for all previous experiments in this project, are rayon-tipped, however the swabs received within SAIKs typically have a cotton tip. Mock cotton-tip swabs were created following processes outlined within the standard operating procedure (34063V1 Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents). Neat semen dilutions covering the range '1/20', '1/50', 1/100', '1/200 and '1/500' were applied to swabs following the addition of approximately three times as many epithelial cells as are normally added to Diff Lysis positive controls. The swabs were dried on a 35°C hot block.

The three semen donors associated with the best detection sensitivity results from Part 5 ('Donor 4', 'Volunteer 1' and 'Volunteer 3') were selected for use in the fabric tape-lifting and excising experiments and also to produce the mock cotton-tip swabs.

Mock samples were processed following either current ER procedures or the proposed method, as described in Part 3. Adaptations specified in Part 6 (section 10.2.) were incorporated, including applying the established 'optimal' incubation conditions. Incubation for these samples was conducted at 15 mins@~30°C, either on a thermomixer set to 1400 rpm, or a heat-block. The reduction in additive water volume (400 µL) was also maintained from Part 6, as an efficiency saving because Phadebas screening is required only infrequently.

Two duplicate samples for each semen dilution level were used, producing a total of 500 mock samples for this experiment, comprising 200 scrapings, 120 tape-lifts, 120 excisions and 60 cotton-tip swabs (see Tables 5 and 6).

Table 29: For each of five semen donors (scrapings); or three donors (tape-lifts, excisions)

Semen dilution	Current ER method		Proposed ER method	
	Thick fabric	**Thin fabric**	**Thick fabric**	**Thin fabric**
1/20	2 samples	2 samples	2 samples	2 samples
1/50	2 samples	2 samples	2 samples	2 samples
1/100	2 samples	2 samples	2 samples	2 samples
1/200	2 samples	2 samples	2 samples	2 samples
1/500	2 samples	2 samples	2 samples	2 samples

Table 30: For each of three semen donors (cotton-tip swabs)

Semen dilution	Current ER method	Proposed ER method
	Cotton-tip swab	**Cotton-tip swab**
1/20	2 swabs	2 swabs
1/50	2 swabs	2 swabs
1/100	2 swabs	2 swabs
1/200	2 swabs	2 swabs
1/500	2 swabs	2 swabs

11.3. Part 7 - Results

Results are show in the tables below:

Table 31: Scraping, Thick and Thin Fabric, Donor 4

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric - Scraping													
1/20	0	<1+	<1+	neg		pos	<1+	neg	<1+	neg		pos	pos
1/20*	<1+	<1+	<1+	neg		pos	2+	neg	1+	neg		pos	neg
1/50	0	0	0	neg		pos	0	neg	<1+	neg		NR	neg
1/50*	<1+	<1+	<1+	neg		pos	<1+	pos	1+	neg		pos	pos
1/100	0	0	<1+	neg		pos	0	neg	0	neg		neg	neg
1/100*	<1+	<1+	<1+	neg		pos	<1+	neg	<1+	neg		neg	pos
1/200	0	0	0	neg		pos	<1+	neg	1+	neg		pos	neg
1/200*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
Thin Fabric - Scraping													
1/20	0	<1+	1+	neg		pos	1+	neg	1+	neg		pos	neg
1/20*	<1+	<1+	2+	neg		pos	1+	neg	1+	neg		pos	neg
1/50	0	0	<1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/50*	0	0	<1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/100	0	<1+	1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/100*	<1+	<1+	1+	neg		pos	0	neg	0	neg		neg	neg
1/200	0	0	1+	neg		neg	0	neg	0	neg		neg	neg
1/200*	0	<1+	1+	neg		pos	<1+	neg	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	<1+	neg	0	neg		neg	neg
1/500*	0	<1+	<1+	neg		neg	0	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Note2: NR = No result

Table 32: Scraping, Thick and Thin Fabric, Donor 5

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric - Scraping													
1/20	0	<1+	<1+	neg		pos	1+	neg	<1+	neg		pos	neg
1/20*	<1+	<1+	<1+	neg		pos	1+	neg	2+	neg		pos	pos
1/50	0	<1+	<1+	neg		pos*	0	pos	0	neg		neg	pos
1/50*	0	<1+	<1+	neg		neg	0	neg	0	neg		neg	neg
1/100	0	<1+	1+	neg		pos	<1+	pos	<1+	neg		pos	neg
1/100*	0	0	<1+	neg		neg	<1+	pos	0	neg		pos	neg
1/200	0	0	<1+	neg		neg	0	pos	<1+	neg		neg	pos
1/200*	0	0	<1+	neg		neg	0	pos	<1+	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	pos	0	neg		neg	neg
1/500*	0	0	<1+	neg		neg	0	neg	0	neg		neg	pos
Thin Fabric - Scraping													
1/20	<1+	1+	2+	neg		pos	1+	neg	1+	neg		pos	neg
1/20*	<1+	2+	2+	neg		pos	1+	neg	1+	neg		pos	neg
1/50	0	<1+	1+	neg		pos	<1+	neg	0	neg		pos	neg
1/50*	<1+	0	<1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/100	0	0	2+	neg		pos	<1+	neg	0	neg		neg	neg
1/100*	0	0	1+	neg		neg	<1+	neg	<1+	neg		pos	neg
1/200	0	0	2+	neg		neg	0	neg	<1+	neg		neg	neg
1/200*	0	0	1+	neg		neg	0	neg	0	neg		neg	neg
1/500	0	0	2+	neg		neg	<1+	neg	0	neg		neg	neg
1/500*	0	0	1+	neg		neg	0	neg	<1+	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 33: Scraping, Thick and Thin Fabric, Volunteer 1

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric - Scraping													
1/20	0	<1+	<1+	neg		pos	2+	neg	<1+	neg		neg	neg
1/20*	0	<1+	<1+	neg		pos	3+	neg	1+	neg		pos	neg
1/50	0	<1+	0	neg		pos	<1+	neg	2+	neg		pos	neg
1/50*	0	<1+	<1+	neg		pos	<1+	pos	3+	neg		pos	pos
1/100	0	<1+	<1+	neg		neg	0	neg	1+	neg		pos	pos
1/100*	0	0	<1+	neg		neg	<1+	pos	0	neg		neg	neg
1/200	0	<1+	0	neg		pos	1+	pos	<1+	neg		pos	pos
1/200*	0	<1+	0	neg		pos	1+	pos	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	neg	<1+	neg		pos	neg
1/500*	0	0	<1+	neg		neg	0	pos	0	neg		pos	neg
Thin Fabric - Scraping													
1/20	<1+	2+	1+	neg		pos	4+	neg	2+	neg		pos	neg
1/20*	<1+	2+	2+	neg		pos	3+	neg	1+	neg		pos	neg
1/50	<1+	<1+	<1+	neg		pos	1+	pos	1+	neg		pos	neg
1/50*	<1+	1+	1+	neg		pos	1+	neg	1+	neg		pos	neg
1/100	0	0	<1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/100*	0	<1+	1+	neg		pos	0	neg	<1+	neg		pos	neg
1/200	<1+	<1+	1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/200*	0	0	2+	neg		neg	<1+	neg	<1+	neg		pos	neg
1/500	0	0	1+	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	2+	neg		pos	<1+	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 34: Scraping, Thick and Thin Fabric, Volunteer 2

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric - Scraping													
1/20	0	<1+	<1+	neg		pos	1+	neg	1+	neg		pos	neg
1/20*	0	<1+	0	neg		pos	1+	neg	1+	neg		pos	neg
1/50	0	0	0	neg		pos	<1+	neg	<1+	neg		pos	neg
1/50*	0	<1+	0	neg		pos	<1+	neg	<1+	neg		pos	pos
1/100	0	0	0	neg		neg	0	neg	0	neg		pos	neg
1/100*	0	0	0	neg		neg	<1+	neg	0	neg		pos	pos
1/200	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/200*	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	1+	neg		neg	0	pos	0	neg		neg	neg
Thin Fabric - Scraping													
1/20	<1+	1+	2+	neg		pos	1+	neg	1+	neg		pos	neg
1/20*	0	<1+	1+	neg		pos	1+	neg	<1+	neg		pos	neg
1/50	0	<1+	2+	neg		pos	0	neg	<1+	neg		pos	neg
1/50*	0	<1+	2+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/100	0	0	1+	neg		pos	<1+	neg	<1+	neg		neg	neg
1/100*	0	0	2+	neg		pos	<1+	neg	0	neg		neg	neg
1/200	0	<1+	1+	neg		pos	0	neg	<1+	neg		pos	neg
1/200*	0	0	1+	neg		neg	0	neg	0	neg		neg	neg
1/500	0	0	1+	neg		neg	<1+	neg	0	neg		neg	neg
1/500*	0	0	1+	neg		neg	<1+	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 35: Scraping, Thick and Thin Fabric, Volunteer 3

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric - Scraping													
1/20	0	0	<1+	neg		pos	<1+	neg	1+	neg		pos	neg
1/20*	0	<1+	0	neg		pos	1+	neg	1+	neg		pos	neg
1/50	0	<1+	0	neg		neg	0	neg	<1+	neg		pos	neg
1/50*	0	0	<1+	neg		pos	1+	neg	0	neg		pos	neg
1/100	0	0	<1+	neg		pos	<1+	neg	0	neg		pos	neg
1/100*	0	0	<1+	neg		neg	<1+	neg	<1+	neg		pos	neg
1/200	0	0	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/200*	0	0	<1+	neg		pos	0	pos	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	pos	0	neg		neg	neg
1/500*	0	0	<1+	neg		neg	0	neg	<1+	neg		neg	pos
Thin Fabric - Scraping													
1/20	<1+	1+	1+	neg		pos	3+	neg	2+	neg		pos	neg
1/20*	<1+	1+	1+	neg		pos	2+	neg	1+	neg		pos	neg
1/50	0	<1+	1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/50*	0	1+	1+	neg		pos	<1+	neg	<1+	neg		neg	neg
1/100	0	0	1+	neg		pos	<1+	neg	<1+	neg		pos	neg
1/100*	0	<1+	1+	neg		pos	<1+	neg	<1+	neg		neg	neg
1/200	0	0	<1+	neg		neg	<1+	neg	0	neg		neg	neg
1/200*	0	<1+	1+	neg		pos	<1+	neg	<1+	neg		neg	neg
1/500	0	0	1+	neg		neg	<1+	neg	0	neg		neg	neg
1/500*	0	0	1+	neg		neg	<1+	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 36: Tape-lift, Thick and Thin Fabric, Donor 4

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Tape-lift													
1/20	0	0	0	neg		neg	0	neg	<1+	neg		neg	neg
1/20*	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/50	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/50*	0	0	<1+	neg		neg	<1+	neg	<1+	neg		neg	neg
1/100	0	0	0	neg		neg	0	neg	<1+	neg		neg	neg
1/100*	0	0	0	neg		neg	0	neg	<1+	neg		neg	neg
1/200	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/500	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
Thin Fabric – Tape-lift													
1/20	0	0	0	neg		neg	0	neg	1+	neg		neg	neg
1/20*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/50	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/50*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/200	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubatior for the Proposed Method

Table 37: Tape-lift, Thick and Thin Fabric, Volunteer 1

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Tape-lift													
1/20	0	0	0	neg		neg	<1+	neg	1+	neg		neg	neg
1/20*	0	0	0	neg		neg	1+	neg	<1+	neg		neg	neg
1/50	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/50*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/200	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/500	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
Thin Fabric – Tape-lift													
1/20	0	0	0	neg		neg	1+	neg	1+	neg		neg	neg
1/20*	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/50	0	0	<1+	neg		neg	<1+	neg	<1+	neg		neg	neg
1/50*	0	0	0	neg		neg	1+	neg	0	neg		neg	neg
1/100	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/100*	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/200	0	0	1+	neg		neg	<1+	neg	<1+	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/500	0	0	0	neg		neg	0	neg	<1+	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 38: Tape-lift, Thick and Thin Fabric, Volunteer 3

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Tape-lift													
1/20	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/20*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/50	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/50*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/100	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/200	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/500	NR	NR	NR	NR	NR	NR	NR	NR	0	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
Thin Fabric – Tape-lift													
1/20	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/20*	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/50	0	0	0	neg		neg	0	neg	<1+	neg		neg	neg
1/50*	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/100	0	0	<1+	neg		neg	0	neg	0	neg		neg	neg
1/100*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/200	0	0	0	neg		neg	<1+	neg	<1+	neg		neg	neg
1/200*	0	0	0	neg		neg	<1+	neg	0	neg		neg	neg
1/500	0	0	0	neg		neg	0	neg	0	neg		neg	neg
1/500*	0	0	0	neg		neg	0	neg	0	neg		neg	neg

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method
Note2: NR = No result

Table 39: Excision, Thick and Thin Fabric, Donor 4

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Excision													
1/20	<1+	<1+	1+	pos	60	pos	1+	pos	2+	pos	40	pos	pos
1/20*	0	<1+	0	pos	60	pos	1+	pos	2+	pos	90	pos	pos
1/50	0	0	<1+	neg		pos	1+	pos	2+	neg		pos	pos
1/50*	<1+	1+	<1+	neg		pos	3+	pos	1+	pos	120	pos	pos
1/100	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100*	0	<1+	<1+	neg		pos	1+	pos	1+	neg		pos	pos
1/200	0	0	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/200*	0	0	1+	neg		pos	1+	pos	<1+	neg		pos	pos
1/500	0	0	1+	neg		pos	0	pos	<1+	neg		pos	pos
1/500*	0	0	0	neg		pos	<1+	pos	<1+	neg		pos	pos
Thin Fabric – Excision													
1/20	0	<1+	1+	pos	60	pos	3+	pos	1+	neg		pos	pos
1/20*	0	<1+	1+	pos	75	pos	2+	pos	1+	neg		pos	pos
1/50	0	0	<1+	neg		pos	2+	pos	1+	neg		pos	pos
1/50*	0	<1+	<1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100	0	1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100*	0	<1+	<1+	neg		pos	<1+	pos	1+	neg		pos	pos
1/200	0	0	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/200*	0	<1+	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/500	0	0	<1+	neg		neg	<1+	pos	<1+	neg		pos	pos
1/500*	0	0	1+	neg		neg	0	pos	<1+	neg		pos	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 40: Excision, Thick and Thin Fabric, Volunteer 1

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Excision													
1/20	0	<1+	0	pos	40	pos	3+	pos	3+	pos	75	pos	pos
1/20*	<1+	1+	0	pos	60	pos	3+	pos	3+	pos	45	pos	pos
1/50	0	<1+	1+	pos	120	pos	2+	pos	<1+	neg		pos	pos
1/50*	0	<1+	1+	pos	100	pos	<1+	pos	1+	neg		pos	pos
1/100	<1+	0	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100*	0	0	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/200	0	<1+	0	neg		pos	<1+	pos	1+	neg		pos	pos
1/200*	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/500	0	0	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/500*	<1+	<1+	1+	neg		pos	<1+	pos	0	neg		pos	pos
Thin Fabric – Excision													
1/20	0	<1+	<1+	neg		pos	1+	pos	3+	neg		pos	pos
1/20*	0	<1+	<1+	pos	75	pos	1+	pos	3+	neg		pos	pos
1/50	0	<1+	<1+	neg		pos	1+	pos	2+	neg		pos	pos
1/50*	<1+	<1+	<1+	neg		pos	1+	pos	3+	neg		pos	pos
1/100	<1+	1+	1+	neg		pos	<1+	pos	3+	neg		pos	pos
1/100*	0	0	<1+	neg		pos	1+	pos	1+	neg		pos	pos
1/200	<1+	<1+	1+	neg		pos	<1+	pos	2+	neg		pos	pos
1/200*	<1+	1+	1+	neg		pos	<1+	pos	2+	neg		pos	pos
1/500	0	<1+	0	neg		neg	0	pos	1+	neg		pos	pos
1/500*	0	0	0	neg		neg	0	pos	1+	neg		pos	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 41: Excision, Thick and Thin Fabric, Volunteer 3

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
Thick Fabric – Excision													
1/20	<1+	1+	<1+	pos	60	pos	3+	pos	2+	pos	120	pos	pos
1/20*	<1+	1+	3+	pos	60	pos	2+	pos	2+	pos	120	pos	pos
1/50	<1+	0	0	pos	105	pos	1+	pos	<1+	neg		pos	pos
1/50*	0	<1+	1+	pos	105	pos	1+	pos	<1+	neg		pos	pos
1/100	0	<1+	0	neg		pos	<1+	pos	<1+	neg		pos	pos
1/100*	0	0	<1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/200	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/200*	0	<1+	<1+	neg		pos	<1+	pos	1+	neg		pos	pos
1/500	0	0	0	neg		pos	<1+	pos	0	neg		pos	neg
1/500*	0	0	1+	neg		pos	<1+	pos	0	neg		pos	neg
Thin Fabric – Excision													
1/20	0	<1+	<1+	neg		pos	1+	pos	4+	neg		pos	pos
1/20*	0	<1+	0	neg		pos	1+	pos	2+	neg		pos	pos
1/50	0	1+	1+	pos	110	pos	1+	pos	1+	neg		pos	pos
1/50*	0	<1+	1+	neg		pos	<1+	pos	1+	neg		pos	pos
1/100	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100*	0	0	<1+	neg		pos	1+	pos	3+	neg		pos	pos
1/200	0	0	<1+	neg		neg	1+	pos	1+	neg		pos	pos
1/200*	0	0	0	neg		neg	1+	pos	<1+	neg		pos	pos
1/500	0	0	<1+	neg		neg	<1+	pos	<1+	neg		neg	neg
1/500*	0	0	<1+	neg		neg	<1+	pos	<1+	neg		pos	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 42: Cotton swab, Donor 4

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
1/20	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/20*	<1+	0	<1+	neg		pos	2+	pos	3+	neg		pos	pos
1/50	0	<1+	<1+	neg		neg	1+	pos	<1+	neg		pos	pos
1/50*	0	0	1+	neg		pos	<1+	pos	<1+	neg		pos	pos
1/100	0	0	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100*	0	0	1+	neg		neg	<1+	pos	1+	neg		pos	pos
1/200	0	0	<1+	neg		neg	<1+	pos	<1+	neg		pos	pos
1/200*	0	0	1+	neg		neg	<1+	pos	<1+	neg		neg	pos
1/500	0	0	<1+	neg		neg	0	pos	1+	neg		neg	pos
1/500*	0	0	<1+	neg		neg	<1+	pos	<1+	neg		neg	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 43: Cotton swab, Volunteer 1

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
1/20	1+	2+	1+	pos	90	pos	3+	pos	3+	neg		pos	pos
1/20*	1+	<1+	<1+	neg		pos	3+	pos	2+	neg		pos	pos
1/50	0	0	0	neg		neg	2+	pos	2+	neg		pos	pos
1/50*	0	<1+	0	neg		pos	3+	pos	2+	neg		pos	pos
1/100	0	<1+	0	neg		neg	2+	pos	2+	neg		neg	pos
1/100*	0	<1+	<1+	neg		neg	2+	pos	2+	neg		neg	pos
1/200	0	0	<1+	neg		neg	1+	pos	0	neg		neg	pos
1/200*	0	0	0	neg		neg	1+	pos	1+	neg		neg	pos
1/500	0	<1+	<1+	neg		neg	1+	pos	1+	neg		neg	pos
1/500*	0	0	0	neg		neg	<1+	pos	<1+	neg		neg	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

Table 44: Cotton swab, Volunteer 3

Semen dilution	Current method								Proposed method				
	Whole sperm	Sperm heads	Epithelial cells	AP	Time (s)	p30	Diff Sperm	Phadebas	Sperm	AP	Time (s)	p30	Phadebas
1/20	<1+	2+	1+	neg		pos	2+	pos	4+	neg		pos	pos
1/20*	1+	2+	<1+	pos	120	pos	4+	pos	4+	neg		pos	pos
1/50	0	<1+	<1+	neg		pos	1+	pos	3+	neg		pos	neg
1/50*	0	<1+	1+	neg		pos	1+	pos	1+	neg		pos	pos
1/100	0	0	0	neg		neg	1+	pos	1+	neg		neg	pos
1/100*	0	0	0	neg		neg	1+	pos	1+	neg		neg	pos
1/200	<1+	<1+	1+	neg		pos	1+	pos	1+	neg		neg	pos
1/200*	0	0	0	neg		neg	1+	pos	<1+	neg		neg	pos
1/500	0	0	0	neg		neg	<1+	pos	<1+	neg		neg	pos
1/500*	0	0	0	neg		neg	<1+	pos	<1+	neg		neg	pos

Note: Samples marked with * were agitated in a thermomixer during incubation for the Proposed Method

For data comparison purposes, the results obtained from the various fabric mock samples and the cotton-tip swabs were collated with 20 relevant results obtained from Part 7 (rayon-tip swabs incubated at 15 mins@~30°C). In total 519 results were included for data analysis. (Note: this was originally 520 samples; however one replicate resulted in a quality failure: '1/500' dilution, Current method, Volunteer 3, tape-lift of thick fabric).

There was a relatively high degree of variability between the results obtained, in terms of detection sensitivities for microscopy, AP, p30 and Phadebas across the different categories of experimental variables. Of note:

- Pronounced variability was seen between results obtained from different semen donors, even among the three donors selected as having given the 'best results' from Part 5.
- Even when keeping most variables constant (same donor, sample substrate, sampling technique and method), there was still a notable degree of inconsistency between results across different semen dilutions. For example, this might mean that although the detection limit is approaching '1/500', the sensitivity limit may be relatively low because positive detection for both replicates has not been able to be achieved at every dilution level.

Substrates and Sampling Techniques

- There was significant variation observed between results obtained from different substrates and particularly notable differences seen between the results of different fabric sampling techniques.

Swabs

  - The results obtained from cotton-tipped swabs were comparable to those from rayon-tipped swabs and both types produced encouraging results for Microscopy and Phadebas detection sensitivities. Rayon-tipped swabs gave superior results to cotton-tip swabs for AP. Note that only a limited number of rayon swabs were tested in this experiment.
  - The results obtained from both types of swab were generally comparable to results from fabric excisions.

Fabrics

  - A comparison of results obtained from the three fabric sampling techniques showed excisions were largely superior to scrapings, and both excisions and scrapings were clearly superior to tape-lifting.
  - Tape-lifts generally produced poor results overall, with no positive detection for AP, p30 or Phadebas for either current or proposed method and no sperm detected with the current microscopy method.

- The variation in results and the poor results obtained from tape-lifting, meant that generally applicable detection sensitivity thresholds could not be established.

Due to the degree of variation, it proved fairly challenging to identify consistent trends in the data, however certain aspects were notable:

Microscopy

- The proposed method performed consistently better than ER microscopy following the current method (see Table 45).
- Diff Lysis microscopy also performed consistently better than ER microscopy following the current method.
- Overall, the proposed method did not perform quite as well as Diff Lysis microscopy.
- Microscopy was the most sensitive screening technique for semen/seminal fluid, with more positive results obtained from microscopy than from either AP or p30.
- All 8 substrates / sampling techniques produced at least some positive results (sperm heads were detected).

Table 45: Collated microscopy results

Microscopy	Rayon Swabs	Scraping Thick	Scraping Thin	Tape-lift Thick	Tape-lift Thin	Excision Thick	Excision Thin	Cotton Swabs	Total
Proposed process better than current ER slide	1	5	1	2	3	3	3	3	21
Proposed process consistent with current ER slide			3	1					4
Proposed process worse than current ER slide			1						1
Proposed process better than current diff slide		3				1	2	2	8
Proposed process consistent with current diff slide	1	1	1	1	2				6
Proposed process worse than current diff slide		1	4	2	1	2	1	1	12

AP

- The current method slightly outperformed the proposed method for the detection of AP, although results for different substrate and sampling techniques were variable (see Table 46).
- Only 4 (of 8) substrates / sampling techniques produced positive results. AP was unable to be detected on any fabric tape-lifts or scrapings.

Table 46: Collated AP results

AP	Rayon Swabs	Scraping Thick	Scraping Thin	Tape-lift Thick	Tape-lift Thin	Excision Thick	Excision Thin	Cotton Swabs	Total
Proposed process better						1			1
No difference		5	5	3	3			1	17
Current process better	1					2	3	2	8

Rayon Swabs produced the best AP results:

- Current method;
  - AP Sensitivity limit was '1/100'
  - AP Detection limit was '1/200'
- Proposed method;
  - AP Sensitivity limit was '1/50'
  - AP Detection limit was '1/50'

P30

- Overall the proposed method performed slightly better than the current method, although most results showed equivalent detection sensitivity between each method.
- 6 (of 8) substrates / sampling techniques produced positive results.

Table 47: Collated p30 results

P30	Rayon Swabs	Scraping Thick	Scraping Thin	Tape-lift Thick	Tape-lift Thin	Excision Thick	Excision Thin	Cotton 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

Excision of Fabrics produced the best p30 results:

- Current method;
  - Best p30 Sensitivity Limit was '1/200' (Thin fabric excision)
  - Best p30 Detection Limit was '1/500' (Thick fabric excision)
- Proposed method;
  - Best p30 Sensitivity Limit was '1/500' (Thick and Thin fabric excisions)
  - Best p30 Detection Limit was '1/500' (Thick and Thin fabric excisions)

- 35.7% (114 of 319) of mock samples required additional water to be added to the p30 device in order to complete the test. This seemed to indicate an intermittent issue which affected a particular batch of p30 test devices. This issue will require future monitoring, and it should be noted that the manufacturer recommends adding 200 µL of solution to the test device as standard.

AP and p30 – comparative detection sensitivity

- Overall for both current and proposed methods, P30 testing outperformed AP testing:
- Current method;
  - **AP pos**, p30 neg: 3 samples
  - AP neg, **p30 pos**: 109 samples
- Proposed method;
  - **AP pos**, p30 neg: 0 samples
  - AP neg, **p30 pos**: 125 samples

Phadebas

- The current method (118 positive results) performed slightly better than the proposed method (111 positive results) for Phadebas detection.
- 6 (of 8) substrates / sampling techniques produced positive results.

Table 48: Collated Phadebas results

Phadebas	Rayon Swabs	Scraping Thick	Scraping Thin	Tape-lift Thick	Tape-lift Thin	Excision Thick	Excision Thin	Cotton Swabs	Total
Proposed process better		2							2
No difference	1		4	3	3	2	2	2	17
Current process better		3	1			1	1	1	7

Rayon and cotton-tipped swabs; Thick and Thin Fabric Excisions produced the best Phadebas results:

- Current method;
  - Best Phadebas Sensitivity Limit was '1/500'
  - Best Phadebas Detection Limit was '1/500'
- Proposed method;
  - Best Phadebas Sensitivity Limit was '1/500'
  - Best Phadebas Detection Limit was '1/500'

Incubation

- Results for the proposed method indicated no significant difference between incubation on the heat block or the thermomixer (see Table 10):
    - For Microscopy and p30 there were more positive detection results using the Heatblock.
    - For AP and Phadebas there were more positive detection results using the Thermomixer.

Table 49: Heatblock vs Thermomixer performance

Incubation 15 mins @30°C	Microscopy	AP	p30	Phadebas	Total
Heatblock - positive results	**93**	5	**70**	54	**222**
Thermomixer- positive results	84	**6**	66	**57**	213
Total Positive results	177	11	136	111	435
All Negative results	83	249	123	149	604

11.4. Part 7 – Discussion

The testing of different substrate and sampling types produced varied results as expected and demonstrated that the proposed method was able to perform adequately across a range of mock samples. The proposed method produced results which were at least comparable to those from the current method for most of the tests performed, with the notable exception of AP testing.

Sperm microscopy results obtained for the proposed method were generally far superior to those obtained from the current method. Note that for microscopy the current method refers to the original ER microscopy, rather than the Diff Lysis slide. 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 substantial, given these methods essentially follow the same process, with minor adaptations.

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 testing produced quite poor results using either method. This was one area where the proposed method did not perform satisfactorily, and this was a consistent trend throughout this project.

For p30 testing, the current and proposed methods produced results which were generally comparable, with the proposed method having a slight advantage in terms of

overall detection sensitivity. P30 testing was demonstrated to be more sensitive than equivalent AP testing across a range of sample types.

Phadebas testing was generally comparable between the current and proposed methods, with the current method having a slight advantage in terms of overall detection sensitivity. Phadebas testing was found to be generally very sensitive for both methods, although it is noted that only one saliva donor has been used for this Project and therefore there may well be physiological variability between individuals which has not been explored here.

Although the included data explored a wide range of variables, the semen samples all originated from a limited pool of volunteers (up to 5 individual donors, depending on the test) who may or may not be truly representative of the normal biological range within the male population. It would be both time and cost prohibitive to test every substrate previously observed and it is not unreasonable to extrapolate the results of testing of one substrate / sample type to others that are similar.

There did not appear to be substantial differences between the results obtained from the rayon-tip swabs as opposed to the cotton-tipped swabs, although AP was found to be slightly more sensitive for rayon-tipped swabs. Rayon-tipped swabs showed the best results of any substrate type for AP detection sensitivity. It should be noted that the data available for these samples was fairly limited (20 rayon-tipped swabs tested as opposed to 60 cotton-tipped swabs).

Although sampling method is typically dictated by the substrate type, given the generally poor results obtained from tape-lifts, it would be difficult to recommend this technique for sampling purposes. Based upon these results, excision seems to be the most effective sampling technique for the successful recovery of detectable levels of semen from fabric samples.

Overall there did not seem to be any advantage conferred by using a thermomixer to agitate samples during incubation and indeed the use of a standard heatblock was associated with slightly better results for Microscopy and p30. The thermomixer is therefore not recommended to be adopted in preference to the heatblock for incubation purposes.

Further work was considered, to investigate whether the positive detection of spermatozoa on microscopy slide was correlated to sufficient DNA being available for extraction, in turn leading to informative (male) DNA profiles being able to be generated. One option considered was that some or all of the experimental mock samples could be quantified, with the Y quant value and total quant values being scrutinised to ensure male DNA is present in the sample and not solely on the microscopy slide. Ultimately, it was decided that the detection of sperm through

microscopy could be safely assumed to indicate sufficient male DNA was present, without the need to interrogate this aspect further at this time.

12. Conclusions

This project was initiated to investigate the observations 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. The degree of difference between the sperm counts was difficult to explain merely as a result of liquid concentration during the Diff Lysis separation step. Although these observations did not affect all samples equally, it drew attention to the fact that the current practice of conducting semen microscopy at ER may be an area for improvement. In addition, a lack of relevant data informing the relative sensitivities of Microscopy and other routine presumptive screening tests also suggested the value of conducting research in this area.

During the course of this project a modified process was implemented for sexual assault casework samples, which involved processing all query semen samples through Diff Lysis extraction, even if the initial ER microscopy was negative for sperm. Subsequent Diff Lysis microscopy was conducted for any apparent sperm negative samples, to confirm initial results before reporting. Microscopy results obtained after Diff Lysis were, at times, incongruent with the sperm count on initial ER slides. This process duplication is considered inefficient and continuing with this redundancy measure indefinitely is undesirable.

Initial investigations into the 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. Despite this, early experimental results did show sperm microscopy conducted at ER to be consistently less sensitive than the same technique conducted at the Diff Lysis stage. Although this finding was not unexpected, it is desirable for microscopy to be optimised for maximum possible sensitivity in order to be able to provide the most informative results.

Attempts to develop a more effective method for ER slide preparation and improve sensitivity were explored, however these were ultimately unsuccessful. An alternative 'proposed method' to replace microscopy at ER was devised, whereby all query semen samples proceed directly to Diff Lysis before conducting microscopy. This proposed method was shown to offer improved sensitivity for sperm microscopy whilst retaining the capacity for presumptive testing through the early addition of water and reservation of supernatants. Further experiments were conducted to optimise the proposed method and to ensure it was robust enough to handle a variety of typical casework sample types.

Throughout this project, microscopy results from the proposed method and Diff Lysis slides have shown consistently superior sensitivity for sperm detection in comparison to ER microscopy results. The proposed method frequently (though not always) produced microscopy results which were equivalent to those obtained from Diff Lysis slides. As the proposed method features essentially the same microscopy process as that which occurs at Diff Lysis, then those results where Diff Lysis slides showed superior sensitivity to the proposed method are not overly problematic. Following the proposed method for microscopy represents an efficiency saving (in time and cost) over current practice involving frequent duplication of labour.

From a reporting perspective, the most critical screening information for query semen samples is provided by sperm microscopy results. The observation of even a single spermatozoon can be reported as confirmatory for semen. There is generally a lower evidentiary value placed on screening test results for seminal fluid (AP /p30), although the ability to report the possible presence of seminal fluid is still very valuable and may be critical depending on case circumstances (e.g. azoospermic males).

The current method of ER microscopy records separate counts for whole spermatozoa (sperm head with attached tail), and sperm heads alone (without tails). The ability to differentiate between whole spermatozoa and sperm heads may potentially provide useful information regarding the time elapsed since deposition, as whole sperm are associated with relatively recent ejaculation. After Diff Lysis extraction, whole sperm are not typically seen and therefore, for Diff Lysis microscopy and the proposed method, only sperm heads are counted. 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.

If microscopy sensitivity can be improved by following the proposed method, then it's more likely that low sperm numbers in a sample will be positively detected, and therefore, in the majority of instances it may be of less concern that seminal fluid screening using p30 cannot provide the same degree of detection sensitivity as microscopy. P30 detection sensitivities were found to be generally equivalent between current and proposed methods.

The results of experimental work conducted in this project demonstrate that p30 is a more sensitive screening tool for the detection of seminal fluid, consistently outperforming AP. There were many results where AP detection sensitivity was quite poor, for both the proposed and current method. Overall the current method gave slightly better AP results than the proposed method.

AP testing has been utilised for seminal fluid screening for many years at Forensic DNA Analysis, in conjunction with p30 testing. When consulted, court reporting scientists were generally of the opinion that the AP test was largely redundant given

that p30 is known to offer a higher degree of specificity and is sufficiently sensitive to effectively screen for seminal fluid. This prompted the question as to whether continuing AP testing for subsamples remains a worthwhile practice moving forward.

The use of AP and p30 together is intended to minimise the possibility of false positives and false negatives occurring when screening for seminal fluid. One such potential false negative for p30 is termed the 'high dose hook' effect, whereby a very high level of PSA may overload the ABAcard p30 test mechanism, resulting in an incorrect negative result being recorded. 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 not expected to preclude the sole use of p30 as a screening tool.

It is important to acknowledge that there are potential cost implications for ceasing the use of AP testing as a standard presumptive screening technique for the detection of seminal fluid, instead relying solely upon p30. In terms of cost per sample, p30 tests are significantly more expensive than AP tests. It may be expected that seminal fluid screening would be required less frequently if the proposed method is adopted for casework, as microscopy will become sensitive enough to positively detect low levels of semen in a greater number of samples, reducing the need to conduct as many p30 tests. The reduction in labour costs this represents is expected to provide an efficiency saving, however this is unlikely to entirely offset the additional expense incurred from the increased use of p30 tests.

13. Recommendations

The following recommendations are made, based on the conclusions above:

1. Implement the proposed workflow for the examination of all samples submitted for semen testing. The process set out in Part 3 should be incorporated into a standard operating procedure, which should also include the adaptations described in Part 6, including the addition of 400 µL of water to allow for potential presumptive testing. Sample incubation is recommended to be undertaken using a standard hotblock set at 15 mins@~30°C.
   P30 testing alone to be used as the standard presumptive screening technique for the detection of seminal fluid in the absence of spermatozoa.
2. Cessation of AP testing as a standard presumptive screening technique for the detection of seminal fluid on sub-samples from swabs, fabrics etc., instead maintaining AP screening solely for the purpose of screening whole items (such as clothing or bedding) for the possible presence of semen stains and subsequent sub-sampling.

14. Abbreviations

AP – Acid Phosphatase

Diff Lysis – Differential Lysis Extraction procedure

ER - Evidence Recovery Team

FRIT - Forensic Reporting and Intelligence Team

GMIDX - GeneMapper®ID-X Software

LOD – Limit of Detection

PP21 - PowerPlex®21

PSA / p30 - Prostate Specific Antigen

QPS - Queensland Police Service

RT – Room Temperature

SAIK - Sexual Assault Investigation Kit

SOP - Standard Operating Procedure

Sperm – Spermatozoa (singular: Spermatozoon)

15. Acknowledgements

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Evidence Recovery Team staff, including special acknowledgements to:
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And thank-you to the donors of the biological material provided for testing.

16. References

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2. The Acid Phosphatase screening test for seminal stains 17186V14
3. Examination For & Of Spermatozoa 17189V15
4. Common Forensic DNA Analysis Terms and Acronyms 23849V12
5. Proficiency Testing in DNA Analysis 24123V11 (Archived)
6. Preparation of DNA Quantification Standards & In-house Quality Controls 25874V7
7. DNA IQ™ Extraction using the Maxwell®16 29344V6 (Archived)
8. Examination of Sexual Cases (Forensic Register) 33798V5
9. Examination of Items (Forensic Register) 33800V3
10. DNA IQ™ Extraction using the Maxwell®16 34044V3

11. Preparation & Testing of Extraction Quality Controls and Testing of Extraction Reagents 34063V2
12. Project #78 Verification of ABA cards p30 test
13. Project #95 Validation of the commercially supplied p30 extraction buffer for use with the ABA card p30 test in the DNA Analysis laboratory
14. Project #136 – to freeze Acid Phosphatase reagent aliquots for use in Acid Phosphatase screening for seminal fluid

17. Appendices

17.1 Appendix 1. Previous interim reports

Project #181 Interim report #1 v1.1 - Allan McNevin, Emma Caunt and Cathie Allen, March 2017

Project #181 Interim report #2 v0.3 - Allan McNevin, Emma Caunt and Cathie Allen, August 2018

Project #181 Interim report #3 v0.3 - Allan McNevin, Emma Caunt and Cathie Allen, August 2018

17.2 Appendix 2. Summary of proposed workflow (Recommendation 1)

1. Exhibits received for semen analysis will be sampled according to the most appropriate method for the sample type (e.g. swabs removed from swabs stick, fabrics excised, scraped or tape-lifted etc.)
2. 400 µL of nanopure water will be added to the sample, vortex mixed and incubated at ~30°C for 15 mins, then vortex mixed again followed by centrifugation for 3 minutes at maximum speed
3. 200µL of supernatant will be removed and stored frozen, sample submitted for differential lysis extraction.
4. All Spermatozoa Fractions will be held post extraction pending examination of the Differential Lysis microscopy slide. All Epithelial Fractions will be processed as per current standard operating procedures (e.g. internal swabs from adults will be processed as "extract and hold").
5. Spermatozoa Fractions with spermatozoa detected microscopically will proceed to DNA quantification and subsequent processing according to existing standard operating procedures.
6. Spermatozoa Fractions where no spermatozoa are detected microscopically will have p30 testing performed on the supernatant retained as noted above. Fractions with no spermatozoa detected microscopically and negative p30 testing will be reported as "semen negative" and no further testing will be conducted. Fractions with no spermatozoa detected microscopically and a positive p30 test result will be reported with the appropriate result line and will proceed to DNA quantification and subsequent processing according to existing standard operating procedures.
7. Any sample determined to require presumptive saliva testing as well as semen testing will undergo extraction with a Retain Supernatant Differential Lysis extraction process. The Supernatant retained from the extraction process will be tested using the Phadebas test as per existing standard operating procedures. The remainder of testing will be as outlined in the steps above.

Kirsten Scott

From: Kirsten Scott
Sent: Tuesday, 4 August 2020 10:15 AM
To: Kylie Rika
Cc: Matthew Hunt; 'Allan McNevin'; Chelsea Savage; Emma Caunt; Paula Brisotto
Subject: Another project document to sign
Attachments: #181 Spermatozoa Microscopy Sensitivity Report.pdf

Kylie,

Given you are not on-site, can you please digitally sign the attached project report (as per Paula's email)? When complete please email back to me.

Thanks
Kirsten

Kirsten Scott PhD BSc(Hons) DipMn GDipEd GCEd
Senior Scientist Quality and Projects

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

[redacted]

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Version history

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1.3	02.07.2020	Allan McNevin	Minor updates
2.0	22.07.2020	Allan McNevin	Incorporated feedback from management team

Document sign off

This document has been **approved** by:

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Allison Lloyd	A/Senior Scientist Intel		03/08/2020
Sharon Johnstone	A/Senior Scientist Reporting 1	[redacted]	04/08/2020
Kylie Rika	Senior Scientist Reporting 2	[redacted] Digitally signed by Kylie Rika Date: 2020.08.04 10:53:43 +10'00'	