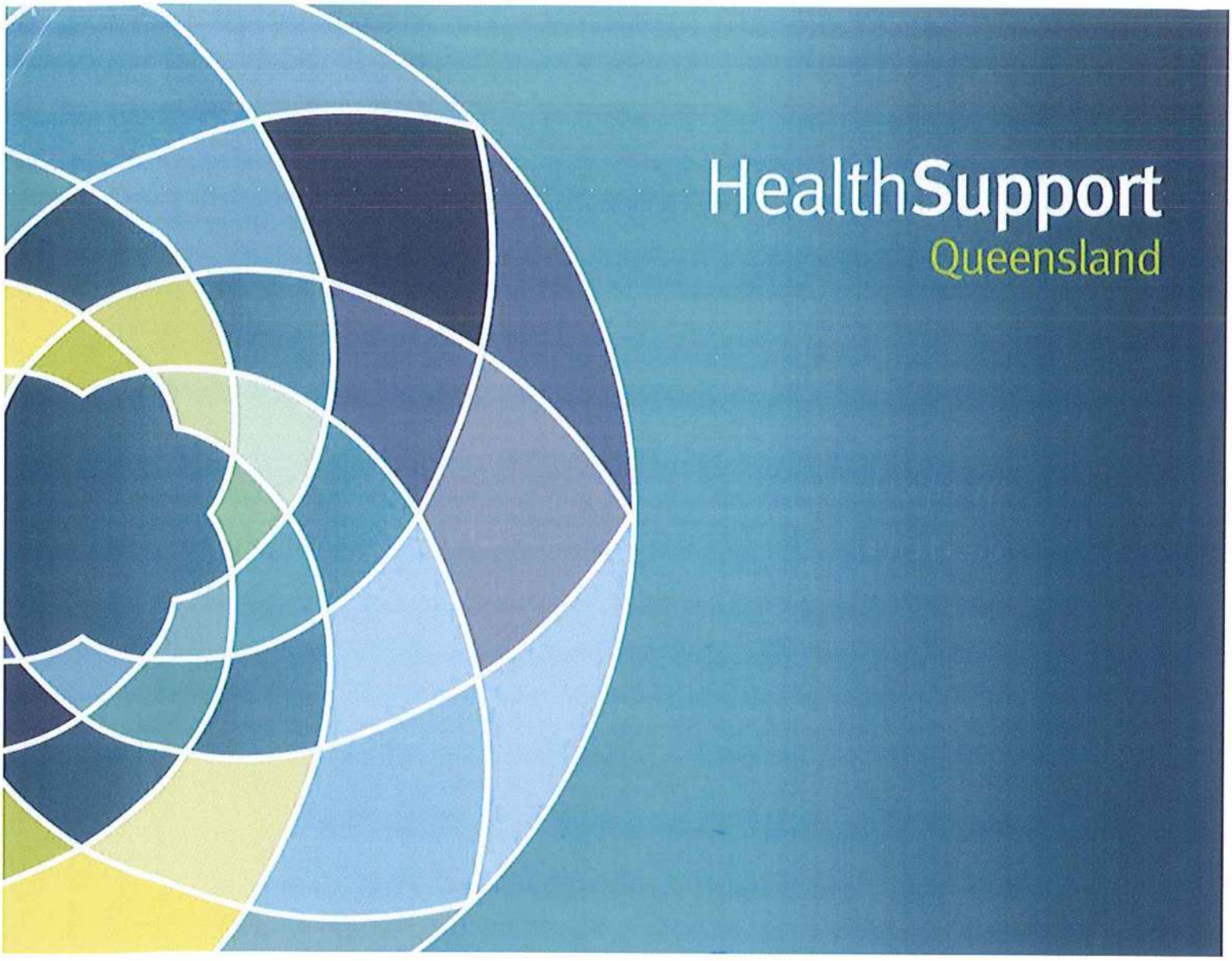

Project Proposal #181

Investigation into the sensitivity of spermatozoa microscopy

August 2016
Allan McNevin, Emma Caunt and Cathie Allen

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

Published by the State of Queensland (Queensland Health), September 2016

For more information contact:
Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Document Details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

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Version history

Version	Date	Changed by	Description
1.0	16/08/2016	Allan McNevin	Document Created.
1.1	28/09/2016	Allan McNevin	Incorporated feedback post review

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist Police Services	[redacted]	13/10/16

The following officers have **endorsed** this documen

Name	Position	Date
Justin Howes	Team Leader FRIT	07·10·2016
Paula Brisotto	Team Leader ER & Q	07-10-2016
Luke Ryan	Senior Scientist Analytical	07-10-2016
Allan McNevin	Senior Scientist ER	06.10.2016
Kirsten Scott	Senior Scientist Q & P	07/10/2016
Sharon Johnstone	Senior Scientist Intel	07/10/2016
Amanda Reeves	Senior Scientist Reporting 1	10.10.2016.
Kylie Rika	Senior Scientist Reporting 2	11/10/2016

Contents

1. Introduction

1.1. Background

Concerns were raised by the Forensic Reporting and Intelligence Team around the difference in spermatozoa microscopy counts observed at the time of examination and the numbers of spermatozoa observed on slides made from the same sample during the differential lysis extraction procedure. Namely, examples where nil or <1+ spermatozoa were observed during item examination and 3+ or 4+ spermatozoa were observed on differential lysis slide microscopy.

Within the Evidence Recovery team, spermatozoa numbers are graded on microscopy using a semi-quantitative scale of 0 (nil seen), <1+ (<10 cells seen on the whole slide, very hard to find), 1+ (10 or more cells

seen, hard to find), 2+ (easy to find); 3+ (very easy to find) and 4+ (abundant). Results from earlier investigations (refer 1.1.1 below) showed examples of casework samples had failed to show the presence of spermatozoa by microscopy during processing in evidence recovery and the presence of spermatozoa from microscopy slides made during differential lysis DNA extraction. The concern therefore is the sensitivity of the original slide microscopy:

i. Is the current suspension method resulting in slides made from overly diluted material such that a sample may be called negative for spermatozoa at the point of examination when there are sufficient numbers present to produce a DNA profile from differential lysis extraction?

ii. Is there a potential problem associated with the slide staining procedure such that spermatozoa are potentially being "lost" and are therefore not visualised on microscopy?

1.1.1. Previous investigations

An initial analysis of a selection of differential lysis slides from samples extracted in 2014 (N=31), 2015 (N=11) and 2016 (N=37) was conducted. Only those samples that had been reported but no statement required of the case, or those samples that had yet to be case managed were included in the selection. Selection was conducted by checking for samples across a number of differential lysis extraction batches in order to capture a range of both evidence recovery and analytical personnel involved in processing. For any sample where the differential lysis slide had not been examined, the slide was stained and microscopy results were recorded.

Results for initial microscopy, DNA quantification of the sperm lysate and differential lysis slide microscopy were tabulated. A total of 79 results were collated. There was a consistent trend towards more spermatozoa observed on the differential lysis slide than what was observed on initial microscopy (N=52), compared to samples where the microscopy was concordant (N=17) and samples where more spermatozoa were seen on initial microscopy (N=10). Average quantification values obtained from sperm lysate samples correlated well with differential lysis extraction slide microscopy, but not so well with initial microscopy. Data available in (G:\ForBiol\AAA Evidence Recovery\Projects and Datamining\2016 comparison of original v diff micro\ 2016 - Diff Lysis slide micro v original micro.xls). There were 7 instances where the original microscopy was negative for spermatozoa but positive from differential lysis microscopy as follows: 2+ (N=2), 3+ (N=2) or 4+ (N=3) were observed from the differential lysis slide. It is worth noting there were also 7 instances no spermatozoa seen on differential lysis slide whilst spermatozoa were observed on original microscopy (all graded at <1+). Results shown in Figure 1 below

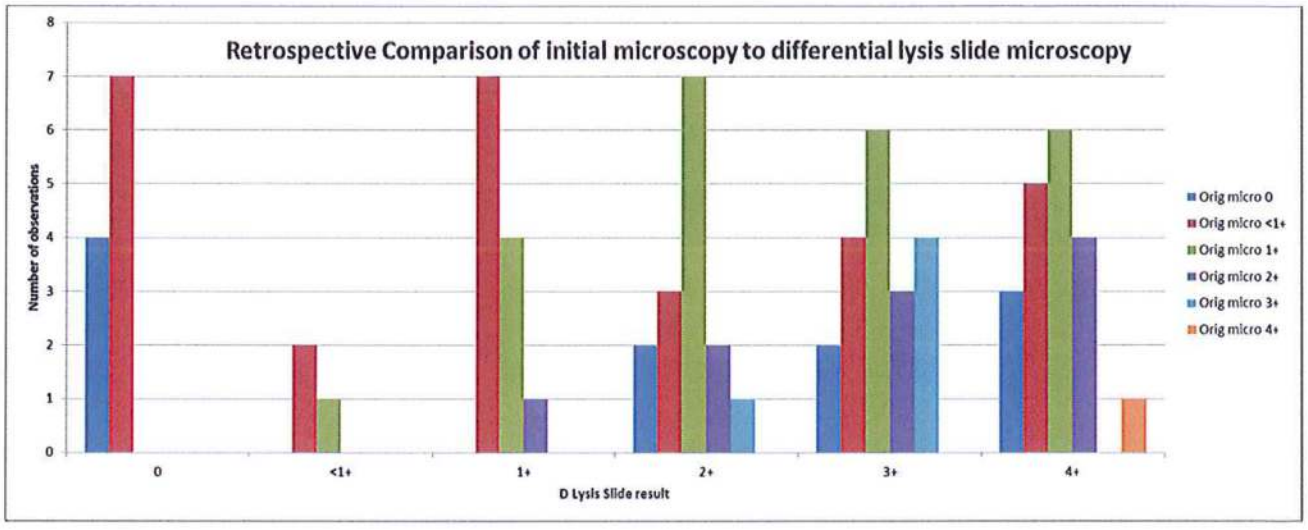


Figure 1 Retrospective data analysis

A review of previously obtained in-house data suggests that current AP and p30 methods have a sensitivity of detecting semen at a dilution of approximately 1/100. A dilution of approximately 1/20 of semen is used for making in-house extraction positive control samples and these samples will yield a microscopy result of approximately 1+ to 2+, with quantification values approximating 0.01 ng/µL (according to positive control log) up to 0.07 ng/µL (according to average positive control results post processing).

2. Purpose and scope

This project aims to investigate the performance of the current method as outlined in standard operating procedures. This project should then inform directions for further investigations.

This project should also then fill a knowledge gap that currently exists within the department. Recording of AP and p30 presumptive testing results compared directly with microscopy results aims to assist in providing indicative information, as there is no current in-house experimental data comparing the sensitivity of sperm microscopy, AP and p30 detection and DNA profiling.

3. Governance

3.1. Project Personnel

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

Senior Project Officer: Emma Caunt, Scientist, Reporting Team

3.2. Decision Making Group

The Management Team and the Senior Project Officer, are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and retained with the original approved Experimental Design.

The Senior Project Officer is included in the Decision Making Group in their capacity as an expert user.

3.3. Reporting

The Project Manager will provide a weekly project status update to the Team Leader, Evidence Recovery and Quality who will in turn advise the Decision Making Group at the Management Team meetings and by exception as required.

4. Resources

The following resources are required for this validation/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)
- 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)

- DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corp., Madison, WI, US)

4.2. Materials

- Sterile 1.5 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)
- Grale HDS SureFrost™ Microscope slides (Trajan Scientific, Milton Keynes, United Kingdom)

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)
- 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)

Forensic DNA Analysis Analytical Staff, Computer and instrument time, as well as bench space in DNA Analysis Analytical Laboratory will also be used in the duration of this project.

5. Methods

5.1. Mock Sample Creation

Mock samples will be created following processes outlined within standard operating procedure 25874V7 Preparation of DNA Quantification Standards & In-house Quality Controls section 5.5 with noted exceptions as follows:

- Instead of dilutions of positive semen control as outlined in the procedure, the following dilutions of neat semen will be used:
  - 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500
- Approximately 3 x the amount of epithelial cells will be added to each swab
- 4 replicates of each semen dilution will be made resulting in 28 mock samples in total.

5.2. Evidence recovery processing

All mock samples will be processed by a single operator following current in-house procedures (17142V12 Examination of items; 171894V13 Examination for & of Spermatozoa), with the exception that regardless of results of microscopy for spermatozoa, samples will also be tested for the presence of AP and p30 (17185V10 Detection of Azoospermic Semen in Casework Samples; 17186V12 The Acid Phosphatase Screening Test for seminal stains).

5.3. DNA extraction

Each swab will undergo a differential lysis extraction process and a slide prepared according to current routine procedure (29344V5 DNA IQ Extraction using the Maxwell 16). The extracts will be held pending further investigations.

6. Experimental Design

6.1. Experiment 1: Investigation of current process

i. Intent

To approximately quantify the difference in the number of sperm observed on microscopy slides made from cell suspensions during the evidence recovery process compared to those made during differential lysis DNA extraction.

Additionally, this experiment may identify the approximate sensitivity of detection of sperm at each of these stages of the process.

ii. Experimental Design

The mock samples created as per 4.1 above will be processed through evidence recovery by a single operator. For differential lysis DNA extraction, the mock samples will be split into two batches, each containing duplicates of each sperm sample dilution. Both batches will be processed by the same operator. Layout of extraction batches is shown in Table 1 below.

Table 1 Extraction batches - Experiment 1

Extraction batch 1		Extraction Batch 2	
Position	Sample	Position	Sample
1	Positive Control	1	Positive Control
2	Negative Control	2	Negative Control
3	1/5 semen dilution	3	1/5 semen dilution
4	1/5 semen dilution	4	1/5 semen dilution
5	1/10 semen dilution	5	1/10 semen dilution
6	1/10 semen dilution	6	1/10 semen dilution
7	1/20 semen dilution	7	1/20 semen dilution
8	1/20 semen dilution	8	1/20 semen dilution
9	1/50 semen dilution	9	1/50 semen dilution
10	1/50 semen dilution	10	1/50 semen dilution
11	1/100 semen dilution	11	1/100 semen dilution
12	1/100 semen dilution	12	1/100 semen dilution
13	1/200 semen dilution	13	1/200 semen dilution
14	1/200 semen dilution	14	1/200 semen dilution
15	1/500 semen dilution	15	1/500 semen dilution
16	1/500 semen dilution	16	1/500 semen dilution

iii. Acceptance Criteria

This experiment has no specific acceptance criteria as it is being used as a baseline upon which further experimentation will be compared.

7. Results and Data Compilation

The results of Evidence recovery presumptive testing, microscopy and differential slide microscopy will be collated and tabulated. This information will formulate decisions on the direction of any further experimentation.

If the Project Team forms the opinion that additional experiments are required before a final assessment can be made, application will be made to the Decision Making Group for a modification to this Experimental Design. The Decision Making Group is responsible for assessing this application and approving or rejecting it.

A final report will be produced which will compile all analyses, conclusion and recommendations. The final report will be prepared by the Project Group.

HealthSupport
Queensland
Forensic and Scientific Services

Project Plan

Stage 2

		Project #:	181
Name/s of Project Staff :	Allan McNevin, Emma Caunt	**Start Date:**	
		Due Date:	
Name Project Team Leader :	Allan McNevin	**Contact Phone Number:**	[redacted]
Technical Reviewer/s	Forensic DNA Analysis management team		
Project Title:	Investigation into the sensitivity of spermatozoa microscopy		
Project type	☐ Administration ☐ IT/LIMS ☒ Laboratory ☐ Data mining/analysis ☐ External Project ☐ Other_____		

Project Background (may include a literature review):

Concerns were raised by the Forensic Reporting and Intelligence Team around the difference in spermatozoa microscopy counts observed at the time of examination and the numbers of spermatozoa observed on slides made from the same sample during the differential lysis extraction procedure. Namely, examples where nil or <1+ spermatozoa were observed during item examination and 3+ or 4+ spermatozoa were observed on differential lysis slide microscopy.

Within the Evidence Recovery team, spermatozoa numbers are graded on microscopy using a semi-quantitative scale of 0 (nil seen), <1+ (<10 cells seen on the whole slide, very hard to find), 1+ (10 or more cells seen, hard to find), 2+ (easy to find); 3+ (very easy to find) and 4+ (abundant). An initial analysis of a selection of differential lysis slides from samples extracted in 2014 (N=31), 2015 (N=11) and 2016 (N=37) showed a consistent trend towards more spermatozoa observed on the differential lysis slide than what was observed on initial microscopy (N=52), compared to samples where the microscopy was concordant (N=17) and samples where more spermatozoa were seen on initial microscopy (N=10). Average quantification values obtained from sperm lysate samples correlated well with Diff Lysis slide microscopy, but not so well with initial microscopy. Data available in (G:\ForBiol\AAA Evidence Recovery\Projects and Datamining\2016 comparison of original v diff micro\ 2016 - Diff Lysis slide micro v original micro.xls). There were 7 instances where the original microscopy was negative for spermatozoa however 2+ (N=2), 3+ (N=2) or 4+ (N=3) were observed from the differential lysis slide. It is worth noting there were also 7 instances no spermatozoa seen on differential lysis slide whilst spermatozoa were observed on original microscopy (all graded at <1+).

The concern is around the sensitivity of the original slide microscopy:

i. Is the current suspension method resulting in slides made from overly diluted material such that a sample may be called negative for spermatozoa at the point of examination when there are sufficient numbers present to produce a DNA profile from differential lysis extraction?

ii. Is there a potential problem associated with the slide staining procedure such that spermatozoa are potentially being "lost" and are therefore not visualised on microscopy?

This project aims to investigate (i) above, as there is no current in-house experimental data comparing the sensitivity of sperm microscopy, AP and p30 detection and DNA profiling. However, if discrepant results are obtained from replicates of the same sample, this project may identify problems related to (ii) above.

A review of previously obtained in-house data suggests that current AP and p30 methods have a sensitivity of detecting semen at a dilution of approximately 1/100. A dilution of approximately 1/20 of semen is used for making in-house extraction positive control samples and these samples will yield a microscopy result of approximately 1+ to 2+, with quantification values approximating 0.01 ng/µL (according to positive control log) up to 0.07 ng/µL (according to average positive control results post processing).

Benefit of Project:

Given that no formal validation of the making of cell suspensions was recorded at the time the procedure was introduced (possibly around 2008, details not found), an investigation into the effectiveness of current procedures will fill the gap in departmental records.

Additionally, the determination of the sensitivity of microscopy and presumptive testing compared to profiling results is worth investigating since this has not been done since the introduction of the PowerPlex21 amplification kit which has a greater level of sensitivity compared to Profiler Plus.

Proposed Methodology:

Mock casework samples will be prepared using a modification of the method used to prepare in-house differential lysis positive control swabs (QIS 25874V7). Initial investigations will consist of:

- Decreasing amounts of semen from a single donor will be applied to a swab in the presence of constant amounts of epithelial cells.
- Proposed semen dilutions are 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500
- Proposed level of Epithelial cells present on initial microscopy will be in the 2+ to 3+ range
- Each swab will be tested using current in-house procedures (17142V12; 171894V13), with the exception that samples that are microscopically positive for spermatozoa will also be tested for the presence of AP and p30 (17186V12; 17185V10).
- Each swab will undergo a differential lysis extraction process and a slide prepared. The extracts will be held pending further investigations.
- Results for sperm microscopy, AP and p30 presumptive tests, and microscopy from each differential lysis slide will all be collated and compared.

Dependant on the results obtained, further investigations will then be proposed; this may involve (for example) testing of samples in an identified critical range, testing of samples from a range of donors and / or various reproducibility or repeatability tests.

Expected Outcome:

- Characterisation of the sensitivity of current procedures as they relate to DNA profiling outcomes. This will fill a gap in departmental records, provide greater insight into current procedures, and may identify an area for procedural improvement.
- Assessment of the sensitivity of the detection of spermatozoa on evidence recovery slides.

Outputs and Project Milestones: (Ensure that the Change Management Milestone Register is filled out I:\Change Management\Change Management Milestone Register.xls)

Description of Outputs/Milestones:	Expected due date:	Completed date:
1.		
2.		
3.		
4.		
5.		

If expected due date/s not met - explanation of reason required:

Project Budget: Prepare using QIS 31052 (and attach to Project Plan)	**Total Project Budget**
	$ Not yet supplied.

Gantt Chart (for large projects): If required, refer to Quality team for help preparing (and attach to Project Plan)

RISK ASSESSMENT:
If a risk is identified: Refer to QIS document 29100 and 29106 for further information on risk identification and management.

Team:	Details of Risk/s Identified	Type of Risk/s:
Evidence Recovery :	Nil identified.	☐ Business Risk ☐ OH&S Signature Line Manager [redacted]
Analytical :	Nil identified	☐ Business Risk ☐ OH&S Signature Line Manager [redacted]

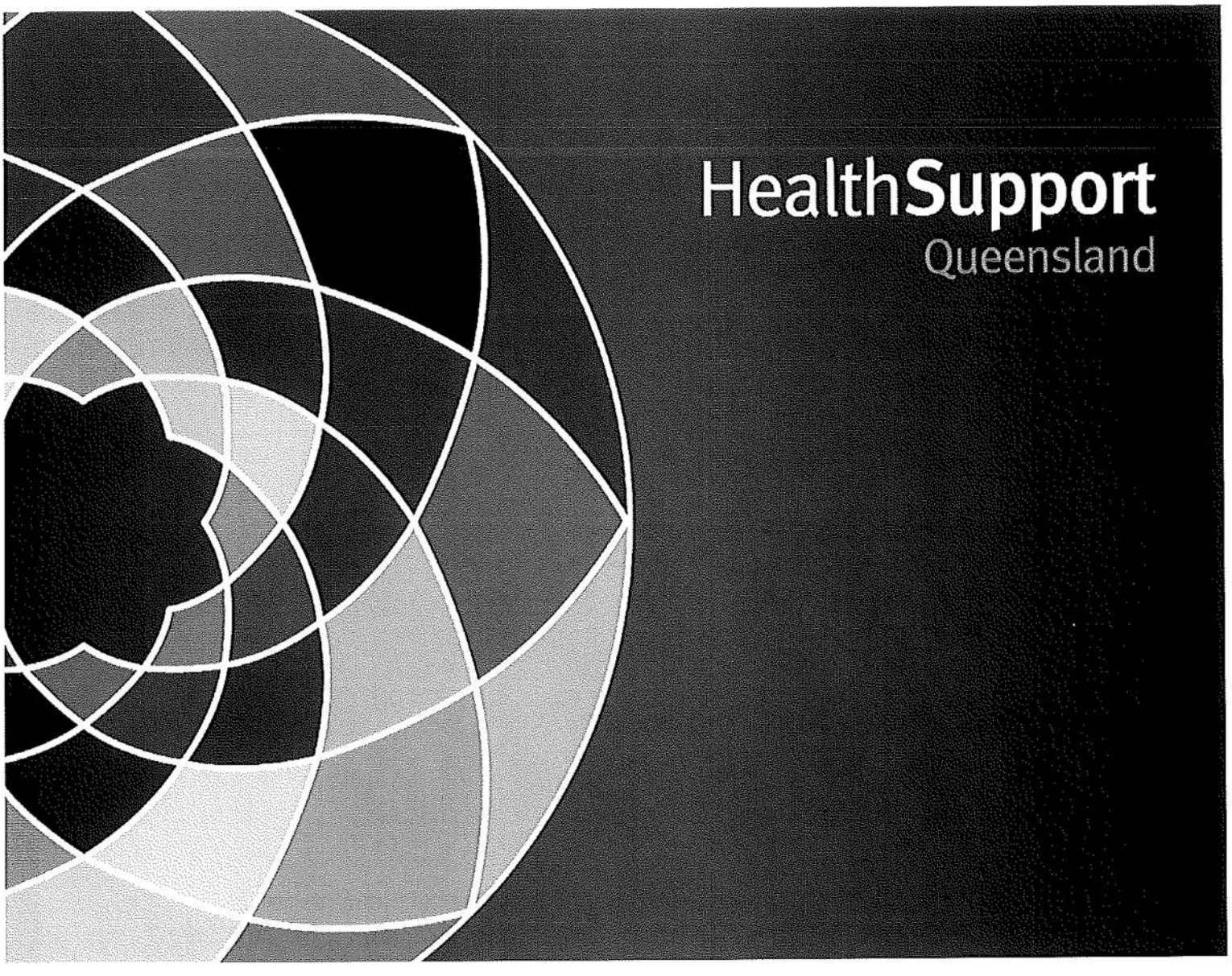
Intel :	No risk to intel	☐ Business Risk ☐ OH&S **Signature Line Manager** [redacted]
Reporting 1:	Nil further risks -	☐ Business Risk ☐ OH&S **Signature Line Manager** [redacted]
Reporting 2 :	This project covers testing to address concerns raised by reporting teams plus extra testing. No risks identified.	☐ Business Risk ☐ OH&S **Signature Line Manager** [redacted]
Quality and Projects (includes OO) :	Given concern with process efficiency only risk is if an assessment of process is not undertaken. This project is an initial assessment to mitigate this risk.	☐ Business Risk ☐ OH&S **Signature Line Manager** [redacted]
Admin :	Nil risk to Admin.	☐ Business Risk ☐ OH&S **Signature Line Manager** [redacted] 12.10.2016.
Team Leader ER &Quality :	Minimal risks as project is initially assessing current processes. Further investigations or projects may be proposed as an outcome of this assessment.	☐ Business Risk ☐ OH&S **Signature Team Leader** PMB [redacted]
Team Leader FRIT :	No risks to this project.	☐ Business Risk ☐ OH&S **Signature Team Leader** [redacted]

		JAH

Project Proposal approved by:			
Signature Team Leader ER and Quality:	[redacted]	**Date:**	13/10/2016
Signature Team Leader FRIT:		**Date:**	12/10/2016
Signature Managing Scientist:		**Date:**	13/10/16 2016 [redacted] 13/10/2016.

Comments:

Please send to Quality Team [redacted] after completion



Project Proposal #181

Investigation into the sensitivity of spermatozoa microscopy – Part 2

April 2017

Allan McNevin, Emma Caunt and Paula Brisotto

Project Proposal #181 - Investigation into the sensitivity of spermatozoa microscopy – Part 2

Published by the State of Queensland (Queensland Health), April 2017

For more information contact:
Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Document Details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

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Title: Senior Scientist – Evidence Recovery
Phone: [redacted]
Email: [redacted]

Version history

Version	Date	Changed by	Description
1.0	16/08/2016	Allan McNevin	Document Created.
1.1	02/05/2017	Allan McNevin	Incorporating feedback from FSS DNA Analysis management team

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Paula Brisotto	A/Managing Scientist Police Services	[redacted]	04.05.2017

The following officers have **endorsed** this documen

Name	Position	Date
Justin Howes	Team Leader FRIT	02/05/2017
Luke Ryan	A/Team Leader ER & Q	02-05-2017
Megan Mathieson	A/Senior Scientist Analytical	02-05-2017
Allan McNevin	Senior Scientist ER	02-05-2017
Kirsten Scott	Senior Scientist Q & P	02/05/2017
Sharon Johnstone	Senior Scientist Intel	03/05/2017
Matthew Hunt	A/Senior Scientist Reporting 1	02/05/2017
Kylie Rika	Senior Scientist Reporting 2	04/05/2017

Contents

1. Introduction

This Experimental Design document outlines additional experiments agreed upon by the Decision Making group after the results of initial experimentation were presented 16-03-2017. Agreement was reached via e-mail vote.

2. Purpose and scope

This is unchanged from the initial proposal.

3. Governance

3.1. Project Personnel

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

Senior Project Officer: Emma Caunt, Scientist, Reporting Team

3.2. Decision Making Group

The Management Team and the Senior Project Officer are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and retained with the original approved Experimental Design.

The Senior Project Officer is included in the Decision Making Group in their capacity as an expert user.

3.3. Reporting

The Project Manager will provide a weekly project status update to the Team Leader, Evidence Recovery and Quality who will in turn advise the Decision Making Group at the Management Team meetings and by exception as required.

4. Resources

The resources required for part 2 of this project are the same as for initial testing.

5. Methods

5.1. Mock Sample Creation

Mock samples will be created following processes outlined within standard operating procedure 25874V7 Preparation of DNA Quantification Standards & In-house Quality Controls section 5.5 with noted exceptions as follows:

- Instead of dilutions of positive semen control as outlined in the standard procedure, the following dilutions of neat semen will be used: 1/50 and 1/100
- Approximately 3 x the amount of epithelial cells will be added to each swab
- Swabs to be dried using hot block set to 35°C rather than temperature outlined in the standard procedure
- 6 replicates of each semen dilution will be made resulting in 12 mock samples in total.

5.2. Evidence recovery processing

All mock samples will undergo standard processing for microscopy for spermatozoa, using a single operator as per current in-house procedures (17142V12 Examination of items; 171894V13 Examination for & of Spermatozoa). At the conclusion of making the microscopy slide, the following processing of the samples will occur:

i. Swab to be transferred to a spin basket and centrifuged for 2 minutes at maximum speed

ii. Transfer supernatant (from step above) and spin basket swab to new tubes, leaving the cell pellet and a small amount of supernatant to be resuspended. A second slide is to be made from the resuspended pellet and read alongside the initial slide.

iii. Cell pellet & swab from spin basket to submitted for DNA profiling as separate samples

5.3. DNA extraction

Each sample (total 24 samples) will undergo a differential lysis extraction process and a slide prepared according to current routine procedure (29344V5 DNA IQ Extraction using the Maxwell 16). The DNA extracts will be held pending further investigations.

6. Experimental Design

6.1. Experiment 1: Investigation of current process

i. Intent

To determine if the sensitivity of Evidence Recovery microscopy can be improved and if so, does this adversely affect the number of spermatozoa present in the DNA extraction. In addition, to determine if spermatozoa are being retained within the swab during Evidence Recovery processing, and if further pre-processing at Evidence Recovery may be required in order to improve microscopy sensitivity.

ii. Experimental Design

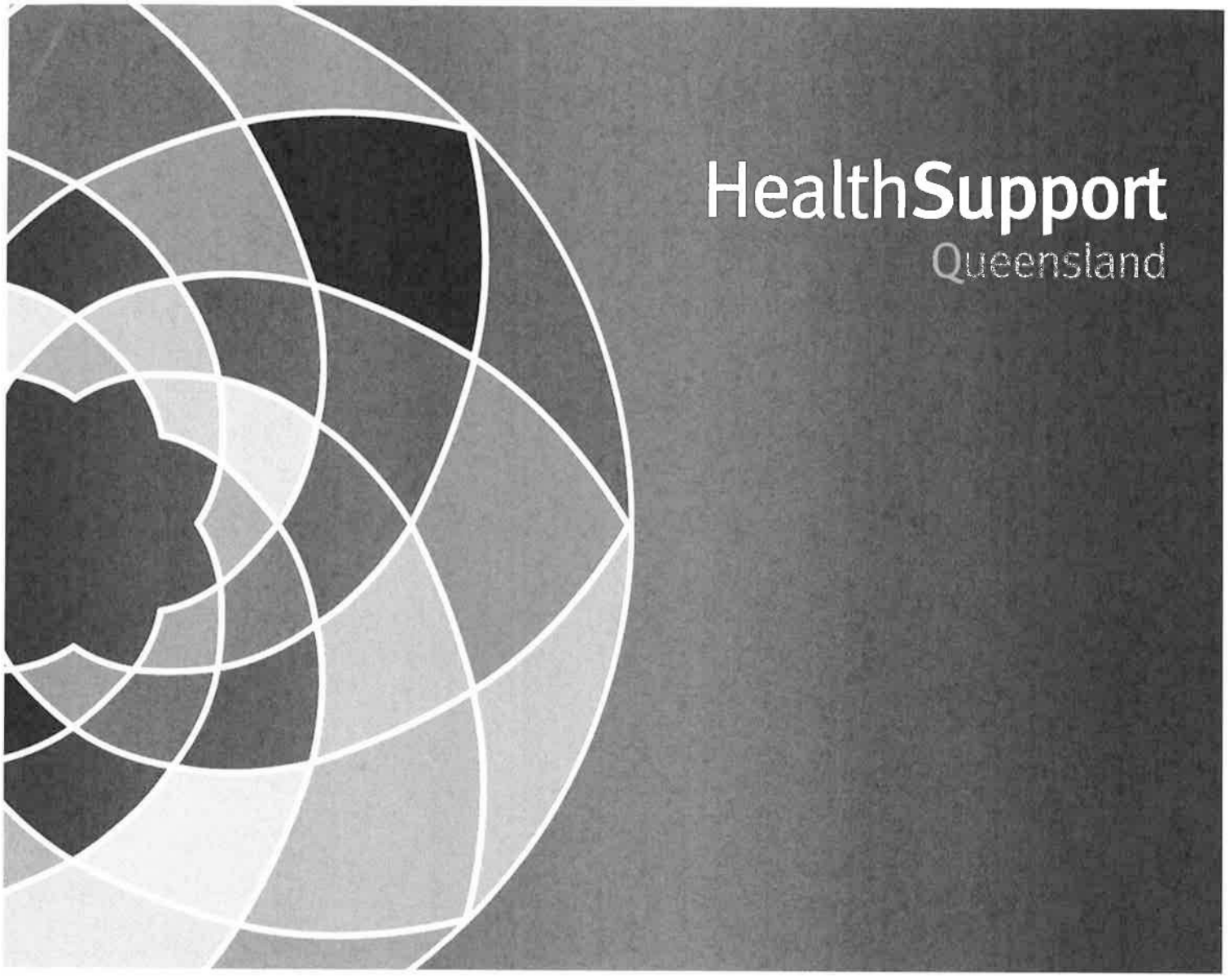
The samples will be processed by the methods outlined above.

iii. Acceptance Criteria

If Evidence Recovery microscopy after spin basket processing is shown to be more sensitive, and does not adversely affect spermatozoa numbers seen during extraction, and spermatozoa are not observed as being retained on the swab material, then the modified method will be considered to be acceptable to proceed to further validation studies.

7. Results and Data Compilation

The results will be collated and presented to the Decision Making Group for further consideration of whether to proceed with additional testing and / or validation and / or workflow design. A further interim report will be produced outlining the findings from this experiment.



Project Proposal #181

Investigation into the sensitivity of spermatozoa microscopy – Part 3

May 2018

Allan McNevin, Emma Caunt and Paula Brisotto

Project Proposal #181 - Investigation into the sensitivity of spermatozoa microscopy – Part 3

Published by the State of Queensland (Queensland Health), May 2018

For more information contact:
Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Document Details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

Contact officer: Allan McNevin
Title: Senior Scientist – Evidence Recovery
Phone: [redacted]
Email: [redacted]

Version history

Version	Date	Changed by	Description
1.0	17/05/2017	Allan McNevin	Document Created.
1.1	22/05/2018	Allan McNevin	Corrected typographical errors, updated acceptance criteria, other minor edits
1.2	29/05/2018	Allan McNevin	Added additional information based on feedback

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist Police Services	[redacted]	05/06/2018.

The following officers have **endorsed** this documen

Name	Position	Date
Justin Howes	Team Leader FRIT	04.06.2018
Paula Brisotto	Team Leader ER & Q	04.06.2018
Luke Ryan	Senior Scientist Analytical	04-06-2018
Allan McNevin	Senior Scientist ER	04-06-2018.
Kirsten Scott	Senior Scientist Q & P	04/06/2018
Sharon Johnstone	Senior Scientist Intel	04/06/2018
Kylie Rika	Senior Scientist Reporting 2	04/06/2018

Contents

1. Introduction

This Experimental Design document outlines additional experiments agreed upon by the Decision Making group after the results of previous experimentation were presented and discussed at both in-person meetings, and via e-mail. Agreement was reached via e-mail discussion and records retained with project documentation.

2. Purpose and scope

This is unchanged from the initial proposal.

3. Governance

3.1. Project Personnel

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

Senior Project Officer: Emma Caunt, Scientist, Reporting Team

3.2. Decision Making Group

The Management Team and the Senior Project Officer are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and retained with the original approved Experimental Design.

The Senior Project Officer is included in the Decision Making Group in their capacity as an expert user.

3.3. Reporting

The Project Manager will provide a weekly project status update to the Team Leader, Evidence Recovery and Quality who will in turn advise the Decision Making Group at the Management Team meetings and by exception as required.

4. Resources

The resources required for part 3 of this project are the same as for previous testing.

5. Methods

5.1. Mock Sample Creation

Mock samples will be created following processes outlined within standard operating procedure 25874V7 Preparation of DNA Quantification Standards & In-house Quality Controls section 5.5 with noted exceptions as follows:

- Instead of dilutions of positive semen control as outlined in the standard procedure, the following dilutions of neat semen will be used: 1/100, 1/200 and 1/500
- Approximately 3 x the amount of epithelial cells will be added to each swab

- Swabs to be dried using hot block set to 35°C rather than temperature outlined in the standard procedure
- 4 replicates of each semen dilution will be made resulting in 12 mock samples in total.

5.2. Evidence recovery processing

Half of the mock samples (two of each dilution) will undergo standard processing for microscopy for spermatozoa, using a single operator as per current in-house procedures (33800V3 Examination of Items (Forensic Register); 17189V14 Examination for & of Spermatozoa), with the exception that AP and p30 testing will be carried out regardless of micro result. The other half of the mock samples will processed using the following workflow:

1. Swabs sampled into a 1.5 mL tube (Note: semen in-tubes are received in a 2.0 mL tube)
2. 650µL nanopure water added to swab head
3. Vortex mix, incubate for 30 mins at room temperature
4. Vortex mix, centrifuge for 2 minutes at maximum speed (note: swab remains in the tube)
5. Transfer 150 µL of supernatant into a new 1.5 mL tube (new barcode, subsample type "SUPNAT" – for any potential, future, 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
6. Samples submitted for processing through routine Differential Lysis DNA extraction

Slides made from routine ER processing, as well as slides made from Differential Lysis extraction (see below) will be read as per SOP (171894V13 Examination for & of Spermatozoa).
(Note: SUPNAT and MISC samples to be stored frozen for 1 week prior to processing to replicate usual maximum routine processing time-frames)

5.3. DNA extraction

Samples undergoing routine Evidence Recovery processing (refer above) will undergo the standard Differential Lysis Retain Supernatant extraction process, samples undergoing modified Evidence Recovery processing (refer above) will be processed through standard Differential Lysis (no retain supernatant) as per SOP (29344V5 DNA IQ Extraction using the Maxwell 16).

5.4. Body fluid presumptive testing

Samples following standard Evidence Recovery testing, and retained "MISC" supernatants will undergo AP and p30 testing following standard laboratory procedures (17186V12 The Acid Phosphatase screening test for seminal stains; 17185V10 Detection of Azoospermic Semen in Casework Samples); retained "SUPNAT" samples (from both modified Evidence Recovery processing, and Differential Lysis Retain Supernatant DNA extraction) will undergo Phadebas testing as per standard laboratory procedures (3398V4 Phadebas test for saliva (Forensic Register))

5.5. DNA Quantification and STR Amplification

All DNA extracts will be processed through DNA Quantification and STR amplification according to standard laboratory procedures (34045V2 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit; 34052V2 Amplification of Extracted DNA Using the PowerPlex21 System)

6. Experimental Design

6.1. Experiment 3: Investigation of alternate process

i. Intent

To investigate the potential for modifying Evidence Recovery processing in order to utilise microscopy performed after initial separation of fractions during Differential Lysis extraction as an effective method for sample screening, and still retain the ability to perform other forms of presumptive body fluid testing

ii. Experimental Design

The samples will be processed by the methods outlined above.

iii. Acceptance Criteria

If the proposed workflow / processing methods provide the same or superior results as the current method, the proposed process will be considered acceptable. This must be true for all results across the same sample dilution – Spermatozoa microscopy, Acid Phosphatase, p30 and Phadebas. If acceptable it will be recommended that a full verification of the changes to procedure be performed. If not acceptable, the Decision Making group will then decide on a way forwards.

7. Results and Data Compilation

The results will be collated and presented to the Decision Making Group for further consideration of whether to proceed with additional testing and / or validation and / or workflow design. A further interim report will be produced outlining the findings from this experiment.

Project Proposal #181

Investigation into the sensitivity of spermatozoa microscopy – Part 4

March 2019

Allan McNevin, Emma Caunt and Paula Brisotto

Project Proposal #181 - Investigation into the sensitivity of spermatozoa microscopy – Part 4

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Document Details

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Version history

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Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist Police Services	[redacted]	08/03/2019

The following officers have **endorsed** this documen

Name	Position	Date
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Allison Lloyd	A / Senior Scientist Intel	08/03/2019
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Contents

1. Introduction

This Experimental Design document outlines additional experiments agreed upon by the Decision Making group after the results of previous experimentation were presented and discussed at an in-person meeting.

2. Purpose and scope

This is unchanged from the initial proposal. The intent of this part (part 4) of the project is to work towards a workflow for the examination of samples for spermatozoa as follows:

1. SAIK / AP pos fabric / semen in-tube is received, examined in Evidence Recovery (ER) and child exhibits registered / sampled and / or subsamples (e.g. scrapings) created

2. ER supernatants collected and stored (as per protocols tested in experiments outlined below)
3. Samples submitted to Analytical – Differential lysis DNA extraction performed and samples held
4. Slides created during Differential Lysis extraction procedure examined in ER
    a. Any samples that are spermatozoa microscopy positive are submitted for quantification and amplification
    b. Any that are spermatozoa microscopy negative are AP / p30 tested (if testing not previously performed by QPS, otherwise accept QPS result)
        i. AP negative samples – NFA
        ii. AP positive / p30 negative samples – NFA
        iii. AP positive / p30 positive submit for quantification and amplification, if Epithelial fraction was originally marked as "Extract and hold", then submit Epithelial fraction for quantification and amplification as well

The workflow noted above has been developed from the results of experimental designs previously developed and tested (Parts 1 to 3 of this project), in particular the testing carried out in Part 3. The main focus of this series of experiments (this being Part 4 of the project) is to further confirm the results of Part 3 and test a range of variables, notably the following:

- Incubation time and temperature
- Substrate type
- Various semen donors

3. Governance

3.1. Project Personnel

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

Senior Project Officer: Emma Caunt, Scientist, Reporting Team

3.2. Decision Making Group

The Management Team and the Senior Project Officer are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and

retained with the original approved Experimental Design.

The Senior Project Officer is included in the Decision Making Group in their capacity as an expert user.

3.3. Reporting

The Project Manager will provide a weekly project status update to the Team Leader, Evidence Recovery and Quality who will in turn advise the Decision Making Group at the Management Team meetings and by exception as required.

4. Resources

The resources required for part 4 of this project are the same as for previous testing.

5. Methods

5.1. Mock Sample Creation

5.1.1. Swabs

Mock samples will be created following processes outlined within standard operating procedure 25874V7 Preparation of DNA Quantification Standards & In-house Quality Controls section 5.5 with noted exceptions as follows:

- Instead of dilutions of positive semen control as outlined in the standard procedure, the following dilutions of neat semen will be used: 1/100, 1/200 and 1/500
- Approximately 3 x the amount of epithelial cells will be added to each swab
- Swabs to be dried using hot block set to 35°C rather than temperature outlined in the standard procedure

5.1.2. Alternate substrates

Mock samples will be created by firstly preparing DNA free fabric using an in-house derived method outlined in SOP QIS 24123V9 Proficiency Testing in Forensic DNA Analysis, Appendix A. Briefly, each piece of fabric under test is firstly washed in a solution of bleach followed by addition of sodium sulphite. This is then followed by 3 rinses in nanopure water to remove bleach and sodium sulfite solution and allowed to dry.

For each fabric sample required, once dried, an area will be marked and the required semen dilution will be added and allowed to be dried. This area will

then be sampled by scraping or tape-lifting according to standard laboratory procedures as outlined in section 5.2 below. The number of samples required is dependant on the number of semen donors able to be found for this project, therefore the exact number of samples to be taken has not been outlined in this document.

5.2. Evidence recovery processing

Two of samples of each semen dilution for each substrate type (refer 6.1 & 6.2 below) will undergo standard processing for microscopy for spermatozoa, using a single operator as per current in-house procedures (33800V3 Examination of Items (Forensic Register); 17189V14 Examination for & of Spermatozoa), with the exception that AP and p30 testing will be carried out regardless of micro result.

Two further samples for each dilution / substrate type will be processed using the following workflow as used in part 3 of this project, with the exception that variations in incubation temperature and time will be used (two test samples for each Variation). Briefly, the modified protocol used in part 3 is as follows:

1. Each substrate to be sampled into a 1.5 mL tube (as per standard examination protocols)
2. 650µL nanopure water added to each sample
3. Vortex mix, incubate (refer 6.1 and 6.2 below for details)
4. Vortex mix, centrifuge for 2 minutes at maximum speed (note: substrate remains in the tube)
5. Transfer 150 µL of supernatant into a new 1.5 mL tube (new barcode, subsample type "SUPNAT" – for any potential, future, 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
6. Samples submitted for processing through routine Differential Lysis DNA extraction

Slides made from routine ER processing, as well as slides made from Differential Lysis extraction (see below) will be read as per SOP (171894V13 Examination for & of Spermatozoa). (Note: SUPNAT and MISC samples to be stored frozen for 1 week prior to processing to replicate usual maximum routine processing time-frames)

5.3. DNA extraction

Samples undergoing routine Evidence Recovery processing (refer above) will undergo the standard Differential Lysis Retain Supernatant extraction process, samples undergoing modified Evidence Recovery processing (refer above) will be processed through standard Differential Lysis (no retain supernatant) as per SOP (29344V5 DNA IQ Extraction using the Maxwell 16).

5.4. Body fluid presumptive testing

Samples following standard Evidence Recovery testing, and retained "MISC" supernatants will undergo AP and p30 testing following standard laboratory procedures (17186V12 The Acid Phosphatase screening test for seminal stains; 17185V10 Detection of Azoospermic Semen in Casework Samples); retained "SUPNAT" samples (from both modified Evidence Recovery processing, and Differential Lysis Retain Supernatant DNA extraction) will undergo Phadebas testing as per standard laboratory procedures (33998V4 Phadebas test for saliva (Forensic Register))

6. Experimental Design

6.1. Experiment 4, part 1: process optimisation testing

i. Intent

To test variations in incubation time and temperature for improvements in AP / p30 test sensitivity without deleterious effects on Phadebas and Microscopy results. Note that, based on results of previous testing, this experimentation is intentionally being performed at or close to the limit of detection of the various tests (AP, p30 / microscopy).

ii. Experimental Design

The samples will be processed by the methods outlined above, with variations in incubation time and temperature as per table below:

Table 1 Time and temperature variations to be tested

Semen dilution	Current ER process	Proposed ER process			
		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

Note: some duplication of testing as covered in part 3 of this project is being performed as there has been a change to the positive control donor, and some variation in results may be observed

If results dictate, further testing with an intermediate dilution (e.g. 1/350) may be conducted.

iii. Acceptance Criteria

The incubation time and temperature tested that provides the same or superior results as the current method will be considered acceptable. This must be true for all results across the same sample dilution – Spermatozoa microscopy, Acid Phosphatase, p30 and Phadebas.

If no results are acceptable, further testing of differing incubation times and / or temperatures may be considered by the Decision Making group.

If an acceptable method has been found, further testing using multiple donors and substrates as outlined in section 6.2 below will be performed.

6.2. Experiment 4, part 2: donor and substrate variation testing

i. Intent

To further test the best protocol identified in section 6.1 above with different semen donors and different substrates / sample types. The substrates / sample types chosen are those most commonly encountered in routine casework testing. It would be both time and cost prohibitive to test every substrate previously observed and it is not unreasonable to extrapolate results of testing of one substrate / sample type to others that are similar.

ii. Experimental Design

The various substrates to be tested are as follows:

- Scraping of a thin fabric (e.g. cotton blend, as is commonly found in underwear, lightweight clothing etc.)
- Scraping of a thick fabric (e.g. bath towel type material)
- Tape-lift of a thin fabric
- Tape-lift of a thick fabric

In addition, it is envisaged that semen from at least 3 different donors be tested across all sample types.

iii. Acceptance Criteria

If the same or superior results are obtained across all tests for all donors compared to previous testing, results will be considered acceptable.

7. Results and Data Compilation

The results will be collated and presented to the Decision Making Group for further consideration of whether to accept results as sufficient for verification of the modified methods or conduct further testing. If results are considered sufficient, a final report will be issued. If further testing is required an interim report will be issued if deemed necessary.