HealthSupport Queensland

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), August 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:

Contact officer: Allan McNevin
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.

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist		

The following officers have **endorsed** this document

Name	Position	Signature	Date
Justin Howes	Team Leader FRIT		
Paula Brisotto	Team Leader ER & Q		
Luke Ryan	Senior Scientist Analytical		
Allan McNevin	Senior Scientist ER		
Kirsten Scott	Senior Scientist Q & P		
Sharon Johnstone	Senior Scientist Intel		
Amanda Reeves	Senior Scientist Reporting 1		
Kylie Rika	Senior Scientist Reporting 2		

Contents

Investigation into the sensitivity of spermatozoa microscopy i
Document Details 2
1. Introduction 3
1.1. Background 3
1.2. Purpose and scope 5
2. Governance 5
2.1. Project Personnel 5
2.2. Decision Making Group 5
2.3. Reporting 5
3. Resources 5
3.1. Reagents 6
3.2. Materials 6
3.3. Equipment 6
4. Methods 7
4.1. Mock Sample Creation 7
4.2. Evidence recovery processing 7
5. Experimental Design 8
5.1. Experiment 1: 8
6. Results and Data Compilation 8
7. References 9
Appendix A – Technical Review Checklist 1

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). 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).

1.2. Purpose and scope

This project aims to investigate the difference in microscopy results between those slides made at the time of item examination and the slides made during the differential lysis DNA extraction process.

This project is investigating 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.

2. Governance

2.1. Project Personnel

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

Senior Project Officer: Emma Caunt, Scientist, Reporting Team

2.2. 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.

2.3. Reporting

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

3. Resources

The following resources are required for this validation/project:

3.1. 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)

3.2. 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)

3.3. 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.

4. Methods

4.1. 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.

4.2. 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 samples that are microscopically positive for spermatozoa 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).

4.3. 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.

5. Experimental Design

5.1. Experiment 1:

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 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.

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.

6. 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.

7. References

1.

Appendix A – Technical Review Checklist

Experiment	Review Task	Reviewer Comment	Reviewer Sign/Date
Experiment 1:			