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).

- Decreasing amounts of semen from a number of different donors will be applied to a swab in the presence of constant amounts of epithelial cells. The number of donors used will depend on the availability, with at least 2 donors required, optimally approximately 5 donors will be utilised
- A number of different donors has been proposed due to expected variation in spermatozoa numbers and levels of AP and p30 activity.
- Proposed semen dilutions are 1/5; 1/10; 1/20; 1/50; 1/100; 1/200; 1/500
- 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).
- Results for sperm microscopy, AP and p30 presumptive tests, DNA quantification and STR profiling of the sperm lysate from differential extraction and microscopy from each differential lysis slide will all be collated and compared.

Dependant on the results obtained, further investigations may then be proposed as a secondary project.

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.

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**
	$

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 :		☐ Business Risk ☐ OH&S **Signature Line Manager**
Analytical :		☐ Business Risk ☐ OH&S **Signature Line Manager**
Intel :		☐ Business Risk ☐ OH&S **Signature Line Manager**

Reporting 1:		☐ Business Risk ☐ OH&S **Signature Line Manager**
Reporting 2 :		☐ Business Risk ☐ OH&S **Signature Line Manager**
Quality and Projects (includes OO) :		☐ Business Risk ☐ OH&S **Signature Line Manager**
Admin :		☐ Business Risk ☐ OH&S **Signature Line Manager**
Team Leader ER &Quality :		☐ Business Risk ☐ OH&S **Signature Team Leader**
Team Leader FRIT :		☐ Business Risk ☐ OH&S **Signature Team Leader**

Project Proposal approved by:			
Signature Team Leader ER and Quality:		**Date:**	
Signature Team Leader FRIT:		**Date:**	
Signature Managing Scientist:		**Date:**	

Comments:

Please send to Quality Team [redacted] after completion