Data Analysis of modified sexual assault process for zero spermatozoa detected at Evidence Recovery

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Introduction

Prior to August 2016, exhibits which gave negative results for spermatozoa and seminal fluid were typically not submitted for DNA testing, using the differential lysis extraction protocol (depending on case circumstances and examination strategy these samples may still be submitted for differential lysis extraction or for cell extraction). In August 2016, the Evidence Recovery Team implemented a modified sample submission process for all samples whereby all samples screened for seminal fluid were submitted for DNA testing, using differential lysis extraction, irrespective of the presumptive screening results (i.e. even those samples for which spermatozoa were not located microscopically and P30 tests were negative were still submitted for DNA testing using a differential lysis extraction). The microscopy slides created during the differential lysis extraction were then read by Evidence Recovery Team scientists for final reporting of spermatozoa detection results.

This modification process was introduced in response to concerns that the initial microscopy conducted during ERT examinations may be detecting fewer spermatozoa than were seen by subsequent microscopy of slides produced after the differential lysis extraction process.

This data analysis examines the set of samples which had no spermatozoa or seminal fluid detected during the initial Evidence Recovery examination, and which were then submitted for differential lysis extraction. The results from this data set will be used to assess the difference in the pre and post August 2016 examination and sample submission strategies based on the final DNA results and attempt to determine what, if any, impact this may have had on the DNA results reported for the case. – this sentence is important and is the purpose – so should be highlighted in some way.

The results from this data set shouldn't be used to:

- extrapolate back to other cases not within this time period and prior to the modified process
- predict the effect on future cases

(as this data doesn't tell the whole story)

In assessing the potential implications for the DNA results reported, relevant aspects of casemanagement were taken into account, including presumptive screening test results, existing examination strategies for different sample types and other results within the case.



Results

Since August 2016, the sample submission strategy was changed for samples which gave negative presumptive screening results for spermatozoa and seminal fluid (i.e. no spermatozoa observed on microscopy and P30 negative test). All samples which were tested for the presence of spermatozoa and seminal fluid were submitted for DNA extraction using the differential lysis protocol, regardless of the presumptive screen results.

During the period, 8 August 2016 to 28 March 2017, there were 738 samples for which the presumptive screening in Evidence Recovery did not identify spermatozoa during microscopy which were submitted for differential lysis extraction. For each of these 738 a microscopy slide was created during the Evidence Recovery examination (the Evidence Recovery slide), and a second slide was created during the differential lysis extraction (the Differential Lysis slide). Both slides were read by Evidence Recovery scientists.

The differences in the methods for creating the Evidence Recovery and Differential Lysis slides are a relevant context to the results of this data mining. Evidence Recovery slides are created by suspending the sample substrate (i.e. swab, scraping, material etc.) 200μ L to 300μ L of nanopure water. This suspension is then vortexed and agitated with a disposable pipette. One drop (using a disposable pipette) of the suspension is then spotted onto a microscopy slide. The Differential Lysis slides are created by taking 3μ L from the male fraction (post separation from the female fraction) during the differential lysis protocol. The total volume for the male fraction when the Differential Lysis slide is created is approximately 50μ L. It is expected that the Differential Lysis slide would be more sensitive (in terms of spermatozoa detection) for one main reason: the Differential Lysis slide is created by sampling 3μ L from a total volume of 50μ L of male fraction, therefore any spermatozoa present in the male fraction are at a higher concentration than the Evidence Recovery suspension which has a total volume of 200μ L.

Table 1 Differential Lysis slide reads for the presence of sperm.

Original ER read	Diff Lysis Slide read	Number of samples	Percentage of Total
0 sperm	0 sperm	591	80.08
0 sperm	<+1 sperm	104	14.09
0 sperm	+1 sperm	36	4.88
0 sperm	+2 sperm	7	0.95

Of these 738 samples, no spermatozoa were observed on both the Evidence Recovery and Differential Lysis slides for 591 samples. This means that for 591 samples, either there were no spermatozoa present, or if they were present they were below the limit of detection for both the Differential Lysis and Evidence Recovery slide preparation techniques.

The remaining 147 slides, for which there were no spermatozoa observed on the Evidence Recovery slide, but where spermatozoa were observed on the Differential Lysis slide, were assessed to determine the impact on final DNA results for that sample. — what about the value of seeing sperm period? For example, it could be highly informative to see sperm even in the absence of a usable DNA result. For example, you don't expect to see sperm at all on a 6 year old girls vaginal swab.

Of the 7 samples for which no spermatozoa were located on the Evidence Recovery slide, but +2 spermatozoa were located on the Differential Lysis slide:

- 6 would have been submitted for differential lysis extraction pre-August 2016 based on a positive P30 result.
- The remaining sample was a vulval sample (690168097) from a SAIK. The vulval sample gave a 3P mixed DNA profile (not yet reported). The high vaginal and low vaginal samples had spermatozoa observed on the Evidence Recovery slides. The high vaginal sample gave a two person mixture which was conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. The low vaginal sample gave a similar result to the high vaginal sample. Therefore failure to submit the vulval sample would not have altered the final result for the SAIK. not really true 3p could have given complainant and two male foreign DNA profiles this could be quite informative compared to a 2p mix of complainant and one male profile.

For the 36 samples which gave no spermatozoa on the Evidence Recovery slide but +1 spermatozoa on the Differential Lysis slide:

- 19 would have been submitted for differential lysis extraction pre-August 2016 based on positive P30 results.
- Seven of these 36 samples would have been submitted for cell extraction rather than differential lysis extraction pre-August 2016. Submission of these seven samples for cell extraction rather than for differential lysis would not have altered the final results for these SAIKs because:
 - o two gave single source profiles consistent with the suspect.
 - o four gave either two/three person mixtures with >100 billion support for suspect contribution.
 - one sample (a perianal SAIK swab) gave a 2P mixture where the known contributor (SAIK complainant) and the suspect were represented. The vulval swab from this SAIK had +1 spermatozoa observed on the Evidence Recovery slide and gave a single source final result consistent with the suspect.
- The remaining ten of these 36 samples would not have been submitted for DNA testing (either by cell or differential lysis extraction protocols) pre-August 2016. Of these ten samples:
 - High vaginal sample (gave a two person mixture which was conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. The second high vaginal, low vaginal, vulval and perianal samples all had spermatozoa detected on the Evidence Recovery slides. The low vaginal and vulval samples gave single source profiles which were consistent with the suspect. The second high vaginal sample gave a two person mixture which was conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. Therefore failure to submit the first high vaginal sample would not have altered the final result for the SAIK.
 - Low vaginal sample (gave a complex final result which was not interpreted. The vulval and rectal samples from this SAIK had spermatozoa detected on the Evidence Recovery slide. The rectal swab gave a single source DNA result which was consistent with the suspect. The vulval gave a complex final result which was not interpreted. Given the results of the rectal sample, and vulval sample, failure to submit the low vaginal sample would not have altered the final DNA results for this SAIK.
 - Low vaginal sample (gave a complex final result which was not interpreted.
 The high vaginal sample from this SAIK was P30 positive and therefore would have been

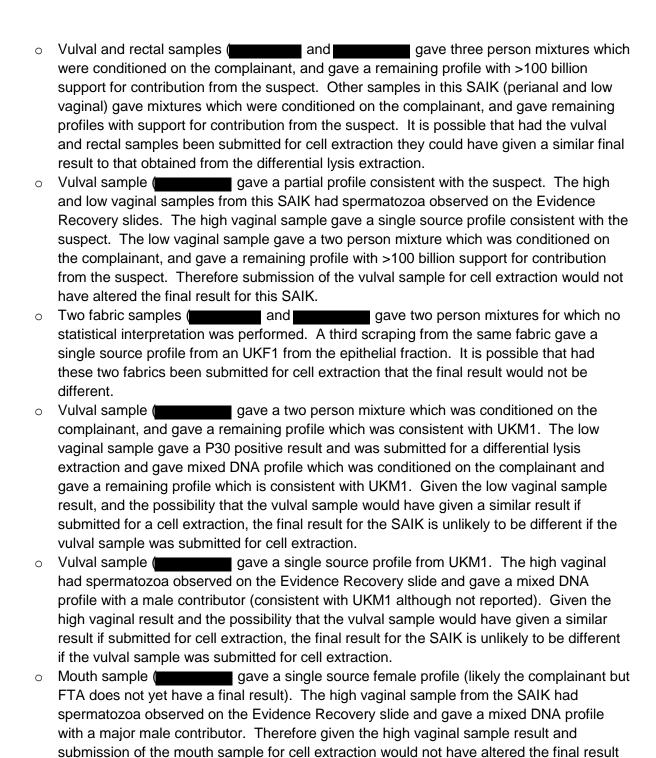
submitted for differential lysis extraction pre-August 2016. This high vaginal swab gave a 2 person mixture with >100 billion support for contribution from the suspect. The perianal swab was also p30 positive and therefore would have also been submitted for a differential lysis extraction pre-August 2016. The perianal swab gave a single source profile consistent with the suspect. The left nipple sample from this SAIK was also submitted for testing and have a 3 person mixture with >100 billion support for contribution from the suspect. Therefore failure to submit the low vaginal sample would not have altered the final result for this SAIK.

- Low vaginal sample (gave a final result which was not interpreted/deconvoluted based on other results from the SAIK. The cervical, high vaginal and posterior fornix all gave 1+ spermatozoa on the Evidence Recovery slide and were submitted for differential lysis extraction (consistent with pre-August process). The cervical sample gave a two person mixture with >100 billion support for contribution from the suspect. Based on this other samples from this SAIK were not interpreted further. Therefore failure to submit the low vaginal sample would not have altered the final result for this SAIK.
- o Low vaginal sample (gave a complex final result which was not interpreted. The high vaginal and vulval samples from this SAIK gave >1+ spermatozoa on the Evidence Recovery slide. The vulval sample gave a complex final result which was not interpreted. The high vaginal gave a 2 person mixture, from which the complainant was excluded. The high vaginal mixture appears to be an 1:1 2 person mixture from two males. No suspect reference samples have been submitted, but if they were they could be compared to this mixture. Therefore failure to submit the low vaginal sample would not have altered the final result for this SAIK.
- Tapelift from inside crotch (gave a complex result for the spermatozoa fraction which was not interpreted. The epithelial fraction also gave a complex result which was not interpreted. Therefore submission of this sample for cellular or differential lysis extraction would not have altered the final DNA result for this sample.
- A piece of fabric (gave a single source profile matching the suspect for the spermatozoa fraction. The epithelial fraction gave a three person mixture with >100 billion support for contribution from the suspect. Submission of this sample for cell extraction would not have altered the final result for this sample (i.e. suspect DNA located), albeit from cells extraction rather than a spermatozoa fraction.
- Low vaginal sample (gave a mixed DNA profile which indicates contribution from a male person, but has not been interpreted based on the high vaginal sample result. The high vaginal sample was P30 positive and therefore submitted for differential lysis and gave a two person mixture which was conditioned on the complainant, and gave a UKM1 remaining profile (n.b. reference sample for suspect does not have a final result). The vulval sample had spermatozoa observed on the Evidence Recovery slide and gave a mixed DNA profile with indications of contribution from a male person, but this result has not been interpreted based on the high vaginal sample result. Therefore based on the high vaginal sample would not have altered the final result for this SAIK.
- High vaginal sample (gave a two person mixture which was conditioned on the complainant and the remaining profile was UKM1 (n.b. offender is unknown). The low vaginal and vulval samples both had spermatozoa observed on the Evidence Recovery slide. The low vaginal gave a similar result to the high vaginal sample and was not interpreted further. The vulval sample was a three person mixture which was conditioned on the complainant, and the remaining profile was not suitable for NCIDD load. Therefore

- based on the low vaginal samples result, failure to submit the high vaginal sample would not have altered the final result for this SAIK.
- A sanitary pad (gave a two person mixture with contribution from a male person (not interpreted further as yet, but apparent major is a male contribution). The high vaginal and rectal samples both had no spermatozoa observed on the Evidence Recovery slide and were P30 negative. The high vaginal sample gave a two person mixed DNA profile which was conditioned on the complainant, and the remaining profile was unsuitable for NCIDD. The rectal sample gave a single source profile which was consistent with the complainant. Therefore failure to submit the sanitary pad for DNA testing would have impacted on the final result of this SAIK, as the sanitary pad was the only sample which gave a profile with a male contribution.

There were 104 samples which for which no spermatozoa were located on the Evidence Recovery slide, but >1+ spermatozoa were located on the Differential Lysis slide (i.e. less than 10 spermatozoa observed on the Differential Lysis slide). The results of these 104 samples should be considered within the context described previously, i.e. that it is expected that the Differential Lysis slides are more sensitive than the Evidence Recovery slide, and that an change in microscopy result from zero spermatozoa detected to between one and ten spermatozoa detected may be representative of this difference in sensitivity. Of these 104 samples:

- 46 would have been submitted for differential lysis extraction pre-August 2016 based on a positive P30 result or examination strategy.
- 39 would have been submitted for cell extraction (rather than differential lysis extraction) pre-August 2016. Of these 39 samples:
 - o 17 samples gave final results which were complex unsuitable for comparison, partial unsuitable for comparison or no DNA detected. Therefore submission for cell extraction would be unlikely to alter the final result for these samples.
 - 8 samples gave final results which were single source and were consistent with an assumed known contributor. Therefore submission of these samples for cell extraction would be unlikely to have altered the final result.
 - Vaginal and anal swab (gave a three person mixed profile which was conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. Given that this sample would have been submitted for cell extraction pre-August 2016, it is likely that a similar result would have been obtained via a cell extraction.
 - Vulval sample (gave a final DNA result which was not interpreted. The high vaginal and low vaginal samples from this SAIK had spermatozoa observed on the Evidence Recovery slide. The high vaginal gave a three person mixture, the low vaginal gave a two person mixture. Both mixtures were conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. Therefore submission of the vulval sample for cell extraction would not have altered the final result for this SAIK.
 - Rectal sample (gave a single source profile consistent with the suspect. The cervical, high vaginal, low vaginal, vulval and perianal samples from this SAIK all had spermatozoa observed on the Evidence Recovery slide and each sample gave a single source profile consistent with the suspect. Therefore submission of the rectal sample for cell extraction would not have altered the final result for this SAIK.



• 19 samples would not have been submitted for DNA extraction (either cell or differential lysis extraction). Of these 19 samples:

for the SAIK.

- 8 samples gave complex unsuitable, partial unsuitable or no DNA detected final results.
 Failure to submit these samples for DNA extraction would not have altered the final result.
- 5 samples gave single source profiles from an assumed known contributor. Failure to submit these samples for DNA extraction would not have altered the final result.
- Low vaginal sample (gave a mixed profile with major contribution from the complainant (which was not interpreted or reported). The high vaginal sample from the SAIK gave a P30 positive result and spermatozoa were detected on the vulval sample on

- the Evidence Recovery slide. The vulval sample gave a two person mixture which was conditioned on the complainant and gave a remaining profile UKM1 which was loaded to NCIDD. Therefore given the vulval result, and the low vaginal result, failure to submit the low vaginal sample for testing would not have altered the final result for this SAIK.
- Endocervix sample (gave two person mixture which was conditioned on the complainant and gave a remaining profile with >100 billion support for contribution from the suspect (this result was the same as for the perianal sample and was not reported via EXH). Spermatozoa were detected on the Evidence Recovery slides for the high vaginal 2, low vaginal, vulval, and perianal samples. The high vaginal gave a similar result to the perianal and was not reported via EXH. The low vaginal and vulval samples both gave single source profiles consistent with the suspect. Given the results of the other samples for this case, and the fact that the endocervix sample was not reported via EXH, failure to submit the endocervix sample for testing would not have altered the final result of the SAIK.
- O High vaginal sample (gave a two person mixture which was conditioned on the complainant and gave a remaining male profile which was compared to two suspects for this case but both were excluded. The cervical sample for this case (which it should be noted had no spermatozoa detected on the Evidence Recovery or Differential Lysis slides) gave a similar result. The low vaginal sample gave a P30 positive result and gave a similar final result to the cervical and high vaginal. Spermatozoa were detected on the rectal sample on the Evidence Recovery slide, but gave a complex final result. Therefore given the results of the low vaginal sample, failure to submit the high vaginal sample for testing would not have altered the final result for this SAIK.
- O High vaginal sample (gave a two person mixture which was conditioned on the complainant and gave a remaining profile with >100 billion support for contribution from the suspect. The vulval had no spermatozoa detected on the Evidence Recovery slide, but which pre-August 2016 would have been submitted for cell extraction, gave a three person mixture which was conditioned on the complainant and gave a remaining profile with >100 billion support for contribution from the suspect. Given that the vulval sample may have given a similar result if submitted for cell extraction (rather than differential lysis) failure to submit the high vaginal sample for testing may not have altered the final result for this SAIK.
- o High vaginal sample (gave two person mixture which was conditioned on the complainant and the remaining profile was used to compare against nominated suspects. The low vaginal sample was P30 positive and gave a three person mixture which was also used to compare against suspects. Given the result of the low vaginal sample, failure to submit the high vaginal is not likely to have altered the final result for this SAIK.
- Fabric sample (gave a two person mixture which had >100 billion support for contribution from the suspect. This was the only result for this sample, however there are a large number of exhibits in this case with >100 billion support for contribution from the suspect. Therefore although failure to submit this sample would have changed the final result of this sample, there are a number of other exhibits in this case linked to the suspect.

Discussion

The aim of this data analysis was to assess the 738 samples which had no spermatozoa or seminal fluid detected during the initial Evidence Recovery examination, and which were then submitted for differential

lysis extraction, and compare these to pre August 2016 examination and sample submission strategies to determine what, if any, impact this (what do you mean by "this") may have had on the DNA results reported for the case as a whole.

738 samples has been considered a sufficiently large dataset for the purposes of drawing some general conclusions, although this relies on the particular cases processed during this period, and therefore sampling variability may show a greater or lesser impact by assessing another dataset. It was beyond the scope of this data analysis to assess slides other than those that were originally zero spermatozoa detected at examination, and were submitted for differential lysis extraction since 8 August 2016. I like this paragraph.

The focus of this data analysis has been largely from a whole case perspective and several results were considered not to be impacted upon because of other samples/ similar results within the case. Assessing results on a whole case basis is standard case management practice, and is a process utilised across all case and sample types. It is acknowledged that the impact on individual samples may be considered significant if semen is not observed at examination, the presumptive screening is also negative and no further action was taken for that sample. The risk if spermatozoa were consequently detected on the differential lysis slide and provided an interpretable DNA result, then potentially a valuable DNA profile for the case may not be obtained. Also don't forget the value of seeing sperm regardless of obtaining a DNA profile or not. What this data analysis shows is that this risk is mitigated when considering the typical case submission as a whole including what the presump expansion explains about the meaning of no sp observed does not equal no sp present. The majority of SAIKs/sexual assault cases contain multiple swabs and items, which provide several opportunities to locate semen and subsequently obtain foreign DNA profile that may support an allegation of sexual assault.

Examination strategies are formulated to try and maximise the chances that even if one sample has no spermatozoa observed and the sample truly contains spermatozoa, then the DNA profile information can be obtained through other means. The presumptive screening for seminal fluid and examination strategies for submitting samples for differential lysis or cell extraction (including but not limited to: submission external swabs/swabs from minors for cell extraction; submitting all areas from an item if one obtains a positive sperm or presumptive result) and also the capacity of STRmix to interpret even mixtures of up to 3 contributors (and including conditioning) all contribute to minimising the overall case impact for a particular sample.

It is acknowledged the slide read at both examination and differential lysis is a detection step, and the sample used to make the slide is a very small amount from the prepared suspension (a drop and 3uL respectively), which is a representation of the spermatozoa that may be present in the sample. For very low levels of spermatozoa, if a second slide is prepared from the sample, lower or higher levels of spermatozoa may be observed, as is expected from sampling variability.

The aim of the differential lysis process is to attempt to separate any spermatozoa from any epithelial cells in order to aid in the interpretation of the DNA profiles obtained. While complete separation of the spermatozoa fractions and epithelial fractions is the ideal, this is not often the case, and carry-over of epithelial cells into the sperm fraction is common. The advantage of using STRmix for mixture interpretation helps mitigate the consequences of failing to obtain the ideal separation of spermatozoa and epithelial fractions, which is the aim for differential lysis. In cases where a sample undergoes a cell extraction and the sample does contain spermatozoa, it is reasonable to assume that this extraction process will extract any DNA present in the sample, including from any spermatozoa present. STRmix will similarly aid in the interpretation of any mixed DNA profiles obtained from this process. — yes but if

you submit through cells and get 4p mix – NFA, whereas you might have got 3pmix in sp frac and SS in epi – you can STRmix the 3pmix.

As described previously, there is a degree of concentration of spermatozoa in the differential lysis process, and the number of spermatozoa present to give a slide read of <+1 is very low (defined as 'very hard to find spermatozoa'), therefore to go from zero to <+1 after differential lysis may not be unexpected. Similarly a slide read of +1 ('hard to find spermatozoa') after differential lysis, following a zero slide read at examination may not be too concerning or necessarily need to be taken as symptomatic of a problem with the examination slide read process.

Where a zero sperm read has produced a +2 sperm read of the slide after differential lysis, then this is harder to rationalise, even allowing for some variation in the subsample taken for the slide, and the differential lysis concentrating step. In this data there were 7 samples of the 738 total which showed this degree of difference ie. Zero to +2 (easy to find spermatozoa) which equates to 0.95% of this sample set. 6 of these samples would have undergone differential lysis extraction based on the presumptive result, and therefore the DNA results would have been unchanged. The one sample remaining was a vulval swab, and would have been submitted for a cell extraction. One sample out of 7 that would have gone through cell extraction and possible comp unsuit result vs a diff lysis with poss 3p and usable result. Within this particular SAIK, the high vaginal and low vaginal swabs both had sperm observed and examination, and provided a DNA profile with a contribution >100 billion for the suspect. Given the reasons listed above, all samples where +2 spermatozoa were detected at differential lysis, the results for the case were not considered to be negatively impacted. Again, just finding suspects DNA may not be the only ideal "result". What about finding DNA that could implicate another person (ie. 3p with diff lysis vs 4p unsuit with cells extn).

Conclusions

Therefore in summary:

- Of the 738 total samples for which no spermatozoa were detected on the Evidence Recovery slide, 591 also had no spermatozoa detected on the Differential Lysis slide.
- 147 of the 738 samples had spermatozoa detected on the Differential Lysis slide (>1+, 1+ or 2+).
- Of these 147 slides, 1 sample (a sanitary pad gave a final result which would not have been obtained pre-August 2016. I.e. the decreased sensitivity of the Evidence Recovery slide (when compared to the Differential Lysis slide) resulted? would have resulted in the sanitary pad sample not being submitted for DNA testing pre-August 2016.

The results of the analysis of this data set have shown that the difference in sensitivity of the Evidence Recovery and Differential Lysis swabs, although acknowledged, has not resulted in a systemic failure (I don't think anyone was ever concerned with there being a systemic failure, rather it being the case that for a small set of samples we are seeing 0sp to 2+sp — why is this? This difference is too big (even though it only relates to a small number of samples) with regards to final reported results. There was one sample in the 738 sample data set which would not have been submitted for DNA testing pre-August 2016, and which gave 1+ spermatozoa on the Differential Lysis swab and a final DNA result consistent with the suspect. This was the only DNA result for this case. Pre-August 2016 this sample would have been reported to the QPS as "Semen not detected" and no further action taken. It should be noted that this presumptive EXH advised the QPS that "Spermatozoa were not observed..." rather than advising

that there were no spermatozoa present. If deemed critical, the QPS could request further processing of this sample.

Therefore, although some individual samples may be negatively impacted as a consequence of the sensitivity of the examination slide process, overall this is considered to be an acceptable risk as it occurs relatively infrequently (which is fine, but why is it happening – proj 181 aims to find out), and from a case perspective the risk is mitigated by the established practices of multiple sample submissions, examination submission and interpretation strategies. This paragraph extrapolates back to all cases which I don't think we can do for reasons previously mentioned.

The results of this study did not demonstrate a systemic failure in the examination of exhibits for seminal fluid. There is a failure in less than 1% of samples. This is a small rate but could have a big impact on the case overall. As long as QPS understand this and that they need to consider that "Spermatozoa were not observed..." does not mean there is no sperm and that about 1% of the time this could be a false negative and they could consider re-testing/further submissions etc... then that is OK. The examination processes described throughout this report, as well as the resulting DNA profile, the assessment of the whole case, and the ability to submit for processing any samples not actioned, aims to mitigate the risk that may arise when spermatozoa is not detected at the examination step. Continuous process improvements are imbedded in Forensic DNA Analysis and are part of our quality management system, and improvements to the examination of sexual assault process will continue, as they will with all processes within the unit, to ensure any risks are mitigated as much as practical. (???)