

# 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 modified process was introduced after it was raised that the initial slide microscopy conducted during ERT examinations may have a lower sensitivity than that slides produced during the differential lysis extraction process.](#)

~~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.

In assessing the potential implications for the DNA results reported, relevant aspects of case-management 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 screening 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 samples 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.) in 200 $\mu$ L to 300 $\mu$ 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 3 $\mu$ L from the male fraction (post separation from the female fraction) during the differential lysis protocol. The total volume for the male fraction from which the Differential Lysis slide is created is approximately 50 $\mu$ 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 3 $\mu$ L from a total volume of 50 $\mu$ 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 200 $\mu$ L. [Suggest could also briefly cover the categorisation of sperm numbers \(how many meant by <1+, 1+ etc.\)](#)

**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

For 591 of these 738 samples, no spermatozoa were observed on either the Evidence Recovery or the Differential Lysis slides. For these results we can infer that 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.

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.
- SUGGEST use our standard wording for all of the below interpretations/stats: for example:
- The remaining sample was a vulval swab sample (██████████ Sug. remove barcodes that could be used to identify case details) from a SAIK. -The

A mixed DNA profile was obtained from this vulval sample swab, spermatozoa fraction which indicated the presence of DNA from 3 contributors, one of whom could be the complainant. Based on statistical analysis it was estimated that the mixed DNA profile obtained is greater than 100 billion times more likely to have occurred if the nominated suspect has contributed DNA, along with the complainant, rather than if he has not.

~~gave a 3P mixed DNA profile (not yet reported). Both the high vaginal and low vaginal samples swabs from this SAIK -had spermatozoa observed on the Evidence Recovery slides. Mixed DNA profiles were obtained from the high vaginal swab, spermatozoa fraction and the low vaginal swab, spermatozoa fraction which each indicated the presence of DNA from 2 contributors, one of whom could be the complainant. Based on statistical analysis it was estimated that the each of these mixed DNA profiles obtained is greater than 100 billion times more likely to have occurred if the suspect has contributed DNA, along with the complainant, rather than if he has not. 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. ?True statement. – the presence of a possible third contributor in the vulval swab may still be considered relevant for the case. Defence could use this evidence of a third person to cast doubt on the complainants story / character – who is this third person? - could this be the true offender etc. - think we should acknowledge that there could be limited impact on the results reported for this SAIK.

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: (As above, too definitive a statement? – A strong contribution from the suspect was obtained for each sample, however there may still be an impact if enough DNA from the female epithelial cells is present following a cell extraction as this could potentially swamp out the contribution of male DNA from the spermatozoa. )
  - The spermatozoa fractions of two of these samples gave single source profiles which consistent with matched the nominated suspect.
  - The spermatozoa fractions of four of the samples gave either two/three person mixtures with >100 billion support for suspect contribution.(Use standard wording..)



- The spermatozoa fraction of the final one sample (was this [REDACTED] (a perianal SAIK swab) gave a 2P mixture where the known contributor (SAIK complainant) and the suspect were represented (use std wording). -The vulval swab from this SAIK had +1 spermatozoa observed on the Evidence Recovery slide and the spermatozoa fraction gave a single source final result consistent with that matched 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-S.frac ([REDACTED]) 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. From the same SAIK, the second high vaginal, low vaginal, vulval and perianal swabs samples (suggest change throughout) 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 ?significantly altered the final results reported for the SAIK.
  - Low vaginal sample SFrac ([REDACTED]) gave a complex final result which was not interpreted. The vulval and rectal samples Sfracs 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 SFrac ([REDACTED]) gave a complex final result which was not interpreted suitable for interpretation. The high vaginal sample SFrac from this SAIK was AP and 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 SFrac ([REDACTED]) gave a final result which was not interpreted/ deconvoluted based on other results from the SAIK, gave a 2person mixture conditioned on the complainant (LR >100billion favouring support for contribution by suspect)– The cervical, high vaginal and posterior fornix Sfracs all gave 1+ spermatozoa on the Evidence Recovery slide and were submitted for differential lysis extraction (consistent with pre-August process). These cervical sample Sfracs each gave a two person conditioned mixtures 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 significantly altered the final reported results for this SAIK.
  - Low vaginal sample SFrac ([REDACTED]) gave a complex final result which was not interpreted (indicates poss 4p). The high vaginal and vulval samples from this SAIK gave >1+ spermatozoa on the Evidence Recovery slide. The vulval sample (Sfrac) gave a complex final result which was not interpreted. The vulval Efrac give a 3p conditioned

The high vaginal (Sfrac) 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. We would be able to talk about probable biological source in court though – given the SFrac is single source and attempting to isolate spermatozoa, then ‘in my opinion highly likely at least some of DNA matching suspect is from semen’.
- Low vaginal sample Sfrac (██████████) gave a 2p conditioned mixed DNA profile >100billion for suspect. which indicates contribution from a male person, but has not been interpreted based on the high vaginal sample result. -The high vaginal sample was AP and P30 positive and therefore submitted for differential lysis and the SFrac gave a two person mixture which was conditioned on the complainant, and gave >100billion favouring contribution by the suspect. 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 the SFrac gave a 3p mixed DNA profile conditioned on the complainant, and gave >100billion favouring contribution by the suspect, with indications of contribution from a male person, but this result has not been interpreted based on the high vaginal sample result. - (Note the Vulval Efrac is yet to be interpreted/reviewed – do not include in report until result finalised) Therefore based on the high vaginal sample Sfrac result, failure to submit the low vaginal sample would not have significantly altered the final result for this SAIK.
- High vaginal sample Sfrac (██████████) 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 Sfrac gave a similar result to the high vaginal sample Sfrac and was has not been interpreted further at this stage. The vulval sample SFrac was a three person mixture which was conditioned on the complainant, and the remaining profile was reported as ‘not suitable for NCIDD load’. The vulval Efrac gave a partial single source DNA profile consistent with the complainant. Therefore based on the low vaginal samples Sfrac result, failure to submit the high vaginal sample would not have significantly altered the final results reported for this SAIK.
- A sanitary pad Sfrac (██████████) **gave a two person mixture (Wait until interp finalised- maybe 3p with repro) 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 Sfrac gave a two person mixed DNA profile which was conditioned on the complainant, and the remaining profile was reported as ‘unsuitable for NCIDD’. The rectal sample Sfrac and Efrac both gave a single source profiles which was were 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 for which no spermatozoa were detected located on the Evidence Recovery slide, but >1+ spermatozoa were detected 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 a 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:
  - 17 samples Sfracs 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 and provide more probative information from for these samples.
  - 8 samples Sfracs 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 significantly altered the final result.
  - Vaginal and anal swab SFrac (██████████) 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. Sfrac gave 2p conditioned, >100 bill for suspect. 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 (██████████) SFrac 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 SFrac 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.
  - Vulval and rectal samples Sfracs (██████████) and (██████████) gave three person mixtures which were conditioned on the complainant, and gave a remaining profile with >100 billion support for contribution from the suspect. (Vulval Efrac = 3p, conditioned, 6300 LR favouring suspect. Rectal Efrac 3p, cond and LR of 2 for susp.) LVS was AP/p30 positive Other samples in this SAIK (perianal and low vaginal sfracs) gave (2p and 3p) mixtures which were conditioned on the complainant, and gave remaining profiles with support for contribution from the suspect. It is possible that had the vulval and rectal samples been submitted for cell extraction they could have given a similar final result to that obtained from the differential lysis extraction.

- Vulval sample (██████████) Sfrac gave a partial profile consistent with the suspect. (Efrac was 3p cond, LR ~140 for susp). The high and low vaginal samples from this SAIK had spermatozoa observed on the Evidence Recovery slides. The high vaginal sample Sfrac gave a single source profile consistent with the suspect. The low 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 submission of the vulval sample for cell extraction would not have altered the final result for this SAIK.
- Two fabric samples (██████████) and (██████████) Sfracs gave two person mixtures for which no statistical interpretation was performed. The Efrac from ██████████ gave a 3p, no statistical interpretation was performed. A third scraping from the same fabric gave a single source profile from an UKF1 from the epithelial fraction. It is possible that had these two fabrics been submitted for cell extraction that the final result would not be different.
- Vulval sample Sfrac (██████████) gave a two person mixture which was conditioned on the complainant, and gave a remaining profile which was consistent with UKM1. Efrac was SS AKC comp't. The low vaginal sample gave an AP/ P30 positive result and was submitted for a differential lysis extraction and Sfrac gave 2p mixed DNA profile which was conditioned on the complainant and gave a remaining profile which is consistent with UKM1. Given the low vaginal Sfrac sample result, and the possibility that the vulval sample would have given a similar result if submitted for a cell extraction, the final result for the SAIK is unlikely to be different if the vulval sample was submitted for cell extraction. (Vulval may have female swamping male if cell ext (given Efrac result)
- Vulval sample Sfrac (██████████) gave a partial single source profile from designated as UKM1. Vulval Efrac has not been interpreted (Matter withdrawn) but indicates a male/female mixture (would require rework to determine # contributors. I wouldn't include this sample in report). The high vaginal swab had spermatozoa observed on the Evidence Recovery slide and the SFrac gave a mixed DNA profile with a male contributor (consistent with UKM1 although not reported). Given the high vaginal sfrac result and the possibility that the vulval sample would have given a similar result if submitted for cell extraction, the final result for the SAIK is unlikely to be different if the vulval sample was submitted for cell extraction.
- Mouth sample Sfrac (██████████) gave a single source female profile (likely the complainant but FTA does not yet have a final result). 2p mix cond compl't, LR supports non-contrib for sus't. Efrac gave SS AKC comp't. The high vaginal sample from the SAIK had spermatozoa observed on the Evidence Recovery slide and Sfrac gave a 2p mixed DNA profile cond on compl't, susp >100bill with a major male contributor. Therefore given the high vaginal sample result and submission of the mouth sample for cell extraction would not have altered the final result for the SAIK.
- 19 samples would not have been submitted for DNA extraction (either cell or differential lysis extraction). Of these 19 samples:
  - 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 Sfrac (██████████) gave a 2p mixed profile cond on compl't 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 and HV Sfracs both 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-High?vaginal result, failure to submit the low vaginal sample for testing would not have altered the final result for this SAIK.

- Endocervix sample Sfrac (██████████) 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 EFrac 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 (2? – need to specify, also applies to HVS 1) gave a similar result to the perianal EFrac and was not reported via EXH. The low vaginal and vulval Sfracs samples both gave single source profiles consistent with the suspect. (Vulval Efrac = 2p cond, LR susp't low support contribn) Given the results of the other samples for this case, and the fact that the endocervix sample was not reported via EXH, (I'd leave this out – only true because no statement request received to date) failure to submit the endocervix sample for testing would not have altered the final result of the SAIK.
- High vaginal sample Sfrac (██████████) 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) Sfrac gave a similar result. The low vaginal sample gave a AP/P30 positive result and the LVS Sfrac gave a similar final result to the cervical and high vaginal Sfracs. Spermatozoa were detected on the rectal sample on the Evidence Recovery slide, but Sfrac gave a complex final result (+ Efrac SS AKC compl't). Therefore given the results of the low vaginal sample Sfrac, failure to submit the high vaginal sample for testing would not have altered the final result for this SAIK.
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- High vaginal sample (██████████) Sfrac 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, Vulval Sfrac 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. Or including the female DNA in the epi fraction may have swamped the male DNA
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- High vaginal sample (██████████) Sfrac 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 AP/P30 positive and the Sfrac gave a three person cond 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. Change in # contributors may impact (as above)
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- Fabric sample (██████████) Sfrac gave a two person mixture which had >100 billion support for contribution from the suspect. (Efrac = complex)-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 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**. Sug. 'due to sampling variation there may have been a greater or lesser impact if another dataset had been assessed. 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.

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 part of 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. There is a-risk that 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. What this data analysis shows is that this risk is mitigated when considering the typical case submission as a whole. 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 of 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 to be expected as a consequence of ~~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 not the case, and carry-over of epithelial cells into the sperm fraction is commonly observed. 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.

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~~ ?inefficiencies in 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 be expected to have been unchanged. The one sample remaining was a vulval swab, which and would have been submitted for a cell extraction. Within this particular SAIK, the high vaginal and low vaginal swabs both had sperm observed ?at and examination, and from these samples provided a mixed DNA profiles were obtained that STRmix gave Likelihood Ratios of greater than 100 billion favouring with a contribution >400 billion for by the suspect. Given the reasons listed above, for all samples within this data analysis where +2 spermatozoa were detected at differential lysis, the DNA profiling results for the case were not considered to be negatively impacted.

## 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 XXXXXXXXXX) 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 has ~~ve~~ shown that the difference in sensitivity of the Evidence Recovery and Differential Lysis swabs, although acknowledged, has not resulted in a systemic failure with regards to final reported results. (I wouldn't include this phrase- don't think a 'systemic failure' of reported results is the concern.)

For a small proportion of sexual assault samples containing low numbers of spermatozoa, the difference in the sensitivity between the ERT and Diff Microscopy methods had previously caused samples to be

reported as semen negative when spermatozoa may have been detectable by microscopy following the differential lysis extraction procedure. As the ERT microscopy was previously used as a key determinant as to which extraction method was employed, and indeed whether the samples were submitted for DNA analysis at all, there is a potential impact on a small subset of reported results. Depending on the case circumstances, the ability to report that semen was detected may or may not be critical, in the context of the allegation. For sexually active adults, confirming the presence of spermatozoa on intimate swabs, may perhaps not be considered as critical as it is for sexual assault allegations involving complainants to whom limited opportunities for the transfer of spermatozoa may make such findings more pertinent (for example minors, the elderly or individuals with disabilities).

In many instances we have found that this issue is mitigated by the common practice of submitting multiple swabs within a typical SAIK, thus increasing the chances of detecting semen. The fact that this data analysis has found that the methods in use prior to August 2016 would have had in only a relatively limited impact on the particular reported results for these SAIKS should be reassuring, however we should continue to strive to eliminate impacts of this type for all cases, and seek to improve the sensitivity of our methods, including by selecting the most effective point in the process to conduct microscopy. It may also be the case that if one swab from a SAIK is affected by this issue, then other swabs in the SAIK could also have an increased chance of being similarly affected, as microscopy examinations are not genuinely independent events. A SAIK containing a High Vaginal Swab with low numbers of spermatozoa may be more likely to also contain a Low Vaginal Swab with low numbers of spermatozoa. The microscopy process is a manual one, which is in a large measure dependent on the technique and ability of the examining scientist. If the slide created from one SAIK swab is affected by an issue which decreases the chance of observing spermatozoa, then this would also tend to affect the chance of detecting spermatozoa on a slide from a second swab of the same SAIK, made by the same scientist.

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, and from a case perspective the risk is mitigated by the established practices of multiple sample submissions, examination submission and interpretation strategies.

The results of this study did not demonstrate a systemic failure in the examination of exhibits for seminal fluid. 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.

(???)<sup>[LR1]</sup>

General points:

Suggest all wording used to describe stats/ interpretations should be as per standard wording for statements - see Procedure for Release of Results SOP 17119.

e.g. "and gave a remaining profile with >100 billion support for contribution from the suspect."

Suggested wording: "Based on statistical analysis it is estimated that the mixed DNA profile obtained is greater than 100 billion times more likely to have occurred if the suspect has contributed DNA along with the complainant, rather than if he has not."

Sug. leave out identifiers (sample barcodes) where discussing interps/case details. ?Could number the samples based on the order in the xls. and refer to Sample 1, 2 etc. within the report – add appendix/notes to explain.

Sug. leave out any interpretations which have not been finalised (reviewed), and may be subject to change.