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Assessment of the Yfiler® Plus PCR amplification kit for the detection of male DNA in semen-negative sexual assault cases

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ABSTRACT

The ability to detect male epithelial cells deposited during digital penetration or penile penetration without ejaculation is limited by the sensitivity of the Y-STR profiling kit. In this study, the relative profiling success of the Thermofisher Yfiler® Plus kit was compared to its predecessor, AmpFlSTR Yfiler®, for 104 semen-negative sexual assault samples from casework at Forensic Science SA, Adelaide, South Australia. Yfiler Plus generated allele information in 25% more samples than Yfiler and gave a higher recovery of informative alleles in all but two samples where detectable male DNA was present. Where a profile was obtained in both kits, 92% of samples gave a higher percentage of informative loci with Yfiler Plus compared to Yfiler. Yfiler Plus also resolved DNA mixtures in 15 samples as compared to 1 sample with Yfiler. Detection of male DNA with the Quantifiler™ Trio DNA Quantification kit was shown to correlate with a successful profiling outcome with Yfiler Plus. The success of profiling with Yfiler Plus was independent of the time elapsed between the alleged offence and the sample being collected, the type of sexual penetration which occurred, and the anatomical origin of the sample.

1. Introduction

The majority of forensic samples encountered in sexual assault casework will be intimate swabs collected from the genital tract of a female complainant in an attempt to recover DNA from a male offender. In scenarios where semen or sperm are absent (*ie*. cases involving digital penetration, penile penetration without ejaculation, or azoospermic (infertile or vasectomised) males), it is known that male epithelial cells from the fingers or penis can be transferred into the vagina and subsequently detected [1–3]. Due to the vast excess of female epithelial cell DNA in these samples, it is extremely unlikely that DNA of an offender would be detected using autosomal profiling. Therefore, Y-chromosome short tandem repeat (Y-STR) profiling, which specifically targets male DNA, has become a significant tool in the forensic investigation of sexual assault crimes. Through the use of Y-STRs, a DNA profile pertaining to a male offender can be successfully generated beyond a 1000-fold excess of female DNA [4–7].

Forensic Science SA (FSSA), Adelaide, South Australia, implemented Y-STR testing in 2007, initially utilising the Thermofisher AmpFlSTR Yfiler® PCR amplification kit (herein referred to as Yfiler). This kit targets 17 loci (with locus DYS385 being multi-copy) and can produce full DNA profiles from 125 pg of male DNA [5,6]. An internal FSSA study showed that whilst Yfiler provided useful information in over 70% of cases where it was employed, useful information was only obtained from 27% of sexual assault samples where semen was absent [8]. A similar finding was reported by McDonald et al. [2] where only 30% of cases involving samples with no spermatozoa yielded informative Y-STR profiles with Yfiler. The low success rate for semen negative samples is likely due to the number of male epithelial cells present in the sample being below the limit of detection of the Yfiler kit.

The Yfiler® Plus kit was released by Thermofisher in 2014 as a successor to Yfiler and was incorporated into forensic casework at FSSA in 2017. In addition to having a higher discrimination power than its predecessor through the inclusion of 10 additional loci (including 7 rapidly mutating markers and a second multi-copy locus) and improved tolerance of inhibitors and degradation [9], Yfiler Plus has also been shown to have a significantly higher sensitivity than Yfiler. Pichrahn et al. [7] detected full Yfiler Plus profiles down to 62.5 pg of male DNA. Ferreira-Silva et al. [10] demonstrated that Yfiler Plus amplified significantly more alleles and generated more complete profiles than Yfiler when tested against 247 samples from sexual assault casework.

This study compared the relative amplification success of the Yfiler and Yfiler Plus kits for 104 sexual assault casework samples where semen was absent to determine if the increased sensitivity of the Yfiler Plus kit translated into more informative profiles in cases of digital penetration and penile penetration without semen. We also investigated

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whether the male DNA quantification value, time elapsed between the offence and the collection of the sample, the type of sexual penetration which occurred, or the anatomical location from where the sample was collected, had any bearing on the result obtained.

2. Materials and methods

2.1. Samples

One hundred and four samples (either individual swabs or a combination of swabs) from 49 sexual assault investigation (SAI) kits submitted for forensic examination at FSSA were included in this study. All swabs gave a negative result to a screening test for acid phosphatase or the ABAcard® p30 antigen test (Abacus Diagnostics) in combination with the absence of spermatozoa by microscopy. Samples which tested positive for the presence of human salivary amylase using the RSID™ Saliva test (Independent Forensics) were also excluded (with the exception of the oral swab where amylase was expected from the sample donor). The anatomical origin, the alleged type of sexual contact, and the time between the alleged offence and collection of the samples were determined from the medical notes contained within the casefile and are given in Supplementary Table 1. All swabs were collected within 44 h of the alleged offence.

2.2. DNA extraction

DNA was extracted using the DNA-IQ™ System (Promega Corporation) as per the manufacturer's instructions using either a manual protocol or on a Multiprobe II liquid handling platform (Perkin Elmer). DNA was eluted in a final volume of 60 µL. DNA extracts were stored frozen at -20 °C between testing with the Yfiler and Yfiler Plus PCR amplification kits.

2.3. DNA quantification

The concentration of male DNA in each DNA extract was determined at the time of Yfiler analysis using the Quantifiler™ Y Human Male DNA Quantification Kit (Thermofisher Scientific). This quantification value was contemporised at the time of Yfiler Plus analysis using the Y target of the Quantifiler™ Trio DNA Quantification kit (Thermofisher Scientific). Quantification tests were performed on an Applied Biosystems 7500 real time PCR machine (Thermofisher Scientific) as per the manufacturer's instructions.

2.4. Y-STR profiling

DNA extracts were profiled using both the Yfiler and Yfiler Plus PCR amplification kits (Thermofisher Scientific) using a GeneAmp 9700 or ProFlex thermocycler (Thermofisher Scientific) in 25 µL reactions with 30 cycles of PCR as per the manufacturer's recommendations. Profiling using the Yfiler Plus kit occurred up to 3 years later than profiling in Yfiler due to the later release and implementation of Yfiler Plus at FSSA.

Only one extract contained sufficient DNA for it to be amplified at the optimal 400 pg DNA template amount (the optimal input DNA was determined by in-house validation of the Yfiler and Yfiler Plus kits at FSSA). The remaining DNA extracts were amplified using the maximum template input volume (10 µL).

PCR products were electrophoresed on an Applied Biosystems® 3130xL Genetic Analyser (Thermofisher Scientific) using 1 µL of PCR product to 9 µL of HiDi™ formamide/GeneScan™ 400HD ROX (for Yfiler) or 10 µL of HiDi™ formamide/GeneScan™ 600 LIZ (for Yfiler Plus). Separated fragments were analysed using GeneMapper® IDX v1.4 software (Thermofisher Scientific) with a peak amplitude threshold of 30 RFU.

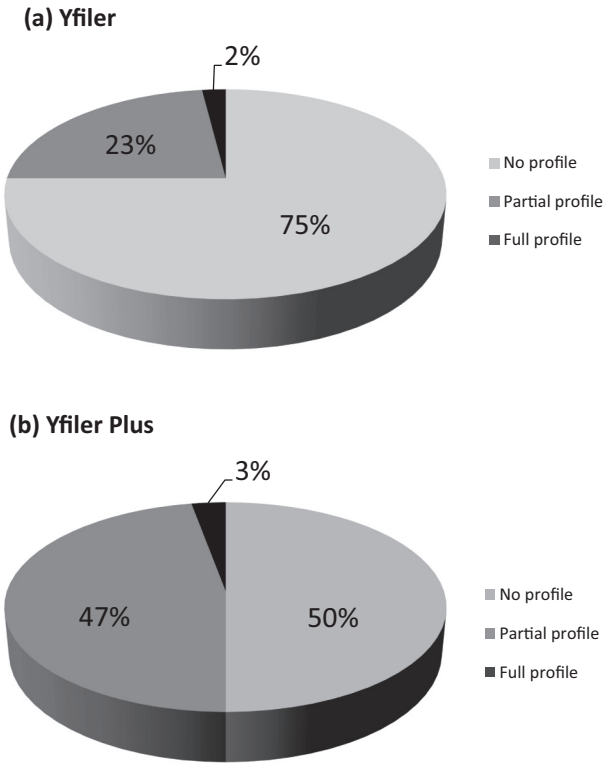

Fig. 1. Percentage of 104 casework samples which gave full profiles, partial profiles or no profiles with (a) Yfiler and (b) Yfiler Plus.

2.5. Ethical considerations

This study was approved by the Forensic Science SA Research Committee. As Yfiler Plus testing had the potential to produce different results to those previously obtained using Yfiler, Yfiler Plus analysis was only performed for sexual assault investigation (SAI) kit samples where police investigations or court proceedings had been withdrawn or cancelled.

3. Results and discussion

3.1. Comparison of Yfiler Plus to Yfiler

The profiling results are summarised in Supplementary Table 1. The mean ± SD number of alleles returned for the 104 samples using Yfiler Plus was 5.8 ± 8.7 (range 0 to 33) as compared to 1.4 ± 3.4 (range 0 to 17) for Yfiler. Some of the profiles obtained were mixtures of DNA from more than one male. The proportion of samples which gave alleles at all loci (full profiles), alleles at some loci (partial profiles) or no alleles (no profile) was determined for each kit and the results are shown in Fig. 1. Alleles were detected in 50% of samples amplified with Yfiler Plus which was double that achieved with Yfiler. The proportion of full profiles increased from 2% to 6% with Yfiler Plus and the proportion of partial profiles increased from 23% to 44%.

For the 52 samples where a Yfiler Plus profile was obtained, the number of alleles recovered in each sample was compared to that achieved with Yfiler (Fig. 2). Of these 52 samples, 28 (54%) yielded a Yfiler Plus profile (defined as the presence of at least one allele) when a Yfiler profile had not previously been obtained. Whilst the majority of these profiles were weak and contained less than 10 alleles, four samples did yield between 15 and 30 alleles which would allow a significant comparison to be made. The remaining 24 samples yielded a DNA profile in both kits but a higher recovery of alleles was achieved

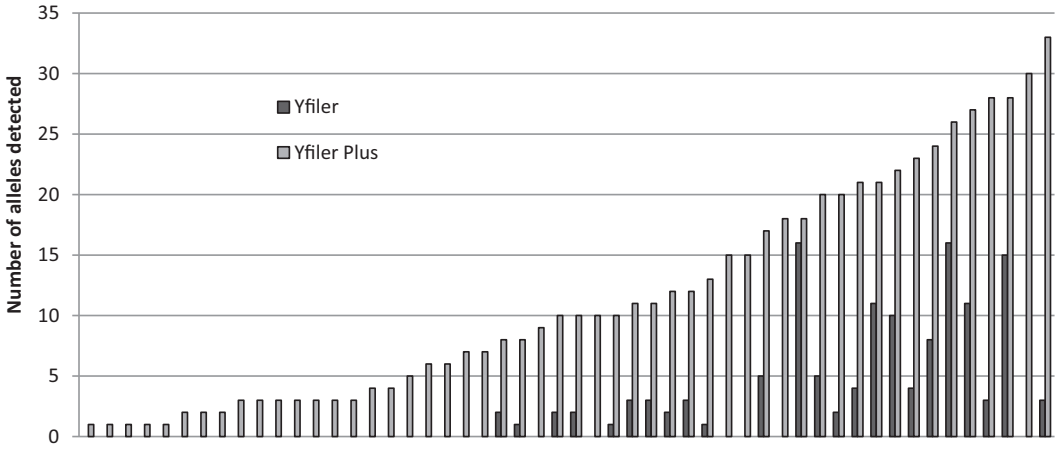

Fig. 2. Comparative number of alleles detected using Yfiler and Yfiler Plus for 52 casework samples where a Yfiler Plus profile was obtained.

with Yfiler Plus.

Two samples which gave detectable alleles in Yfiler (2 alleles and 9 alleles) did not give a profile in Yfiler Plus. The time lapse between Yfiler and Yfiler Plus testing was 21 months for one sample (with 2 alleles) and 26 months for the other (with 9 alleles). Male DNA was not detected in either sample using the Quantifiler Y Human DNA Quantification Kit or Quantifiler Trio at the time of Yfiler or Yfiler Plus analysis. Therefore, it is possible that the very low amount of male DNA that was present initially and detected using Yfiler, had degraded between re-testing with Yfiler Plus. The alleles detected initially with Yfiler testing can be explained by the suspect's reference profile in each case so contamination of the Yfiler result is not likely.

The number of alleles recovered in a profile is a critical factor when comparing to a known reference sample. However, as Yfiler Plus has 10 more loci than Yfiler, an increase in the number of recovered alleles is not necessarily a true indicator of superior kit performance. To determine the overall profiling success of each kit, the percentage of informative loci was compared. An informative locus was defined as a locus where any allelic information was detected. The average percentage of informative loci across all 104 samples for Yfiler Plus was 20.5% as compared to 8.4% for Yfiler. For the 24 samples where a profile was obtained in both kits, 22 samples (92%) gave a higher percentage of informative loci with Yfiler Plus compared to Yfiler (Fig. 3). The increase in the percentage of informative loci for these 22 samples ranged from 8% to 81% with an average of 34%. One sample gave a full profile in both kits and one sample showed a reduction in the percentage of informative loci from 100% with Yfiler to 60% with Yfiler Plus. The sample which gave a reduction in informative loci with Yfiler Plus originated from an anal swab and demonstrated over a 100-fold reduction in the amount of available DNA between the time of Yfiler and Yfiler Plus analysis. Either physical loss or degradation of DNA in this sample may account for this result. Despite this reduction, an extra two alleles were available for comparison with Yfiler Plus and it also detected a second contributor to the profile.

Of the 104 samples tested, only one mixed DNA profile was detected with Yfiler which appeared to consist of DNA from two contributors. When this sample was re-tested with Yfiler Plus, the number of contributors increased to three. Yfiler Plus detected a further 14 mixed DNA profiles in the remaining sample cohort, 13 of which consisted of two contributors and 1 which consisted of three contributors (and yielded either single source or no profiles with Yfiler).

The superior sensitivity of contemporary Y-STR kits (Yfiler Plus or Promega PowerPlex® Y23) to Yfiler for the detection of male DNA across a broad range of exhibits in sexual assault casework was first reported by Ferreira-Silva et al. [10]. In our study, we also demonstrate this specifically in respect to semen-negative sexual assault cases where male epithelial cells are the intended target. Not only does Yfiler Plus result in more alleles being obtained in no semen cases for inclusionary or exclusionary purposes, it also better detects the number of male contributors to a mixed profile. From this it is clearly evident that contemporary Y-STR kits provide more informative and probative profiles than their predecessors in cases involving digital penetration and penile penetration without ejaculation.

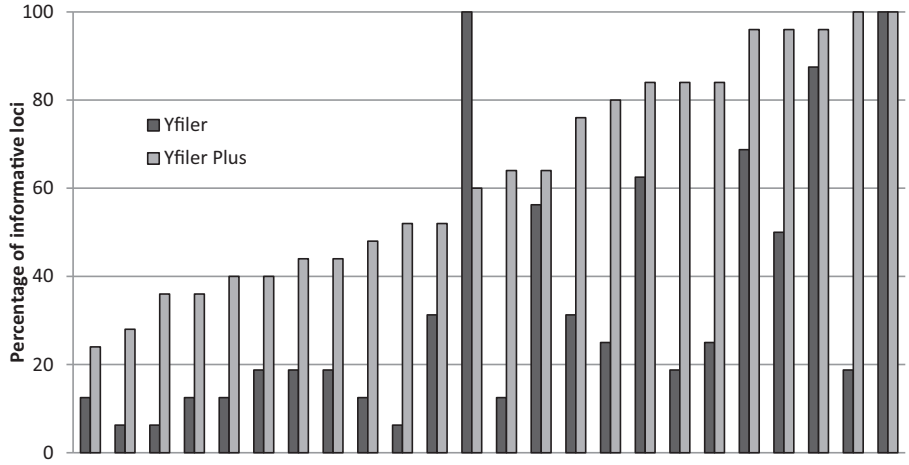

Fig. 3. Percentage of informative loci for Yfiler and Yfiler Plus for 24 casework samples where a Y-STR profile was obtained in both kits.

3.2. Relative sensitivity of Quantifiler Trio and Yfiler Plus

Of the 104 samples tested, 38 gave a Y target value using Quantifiler Trio. These values ranged from 0.0001 to 0.53 ng/μL. The remaining 66 samples had an undetectable Y target. Of the 38 samples where male DNA was detected using Quantifiler Trio, 35 (92%) produced a profile in Yfiler Plus. The number of alleles recovered ranged from 1 to 33 with an average of 15.3 (some profiles were mixtures of more than one male). For the 66 samples where male DNA was not detected using Quantifiler Trio, 17 (26%) produced a Yfiler Plus profile. The number of alleles recovered was much lower and ranged from 1 to 10 with an average of 3.8. When considering samples where the maximum volume (10 μL) of DNA template was added to the PCR, the number of Yfiler Plus alleles obtained was moderately correlated with the concentration of DNA in the extract ($R^2 = 0.39$, least squares regression analysis).

The ability to generate a Yfiler Plus profile in the majority of samples with quantifiable male DNA show that Quantifiler Trio and Yfiler Plus have similar limits of detection. In the absence of detectable male DNA with Quantifiler Trio, the likelihood of generating a Yfiler Plus profile suitable for comparison purposes is low. For this reason, the presence or absence of detectable male DNA in a DNA extract is a useful indicator for triaging samples for further testing in Yfiler Plus. This may lead to increases in laboratory efficiencies and cost savings when these types of samples are encountered.

3.3. Effect of time since offence on Yfiler Plus results

The time between the alleged offence and the collection of the SAI kit samples was available for 97 of the 104 samples and ranged from 5 to 44 h. The number of alleles detected with Yfiler Plus was compared to the elapsed time and the results are presented in Fig. 4. Least squares regression analysis showed that there was virtually no correlation between the number of Yfiler Plus alleles detected and the elapsed time since the offence and the samples being collected ($R^2 = 0.0012$).

Numerous studies have shown that time since intercourse has a direct effect on the ability to recover spermatozoa from the female genital tract [11–14]. Factors that facilitate the loss or degradation of spermatozoa include the activities of the female (*eg.* bathing, douching, physical activity), natural drainage, and the vaginal environment. The persistence of male epithelial cells in the vaginal tract has not been studied and it is therefore not known if their persistence would differ from spermatozoa but it is likely that the mechanisms for loss and/or degradation would be similar. Therefore, it is assumed that the reason for the lack of correlation between the time since offence and the number of Yfiler Plus alleles detected in this study is due to other factors. For example, the number of male epithelial cells initially deposited may be highly variable due to differences in the length, force and friction of penetration. It is also likely that any male epithelial cells deposited will be present in significantly lower levels than spermatozoa regardless of the time since the offence. Therefore, the efficacy of the sampling technique and downstream DNA extraction will have greater impact on the ultimate recovery of Yfiler Plus alleles. As this study uses casework samples, it is possible that the absence of Yfiler Plus alleles in a proportion of samples may be due to false allegations of rape or inaccuracy in reporting the time of the offence by the complainant. In combination, all these factors may bias the data and prevent any correlation being made.

Importantly, what this study does demonstrate is that it is possible to obtain full Yfiler Plus profiles from the female genital tract up to 44 h following an offence where semen is not deposited. This has not previously been demonstrated with Yfiler Plus. A study by Sween et al. [3] reported one example of a full PowerPlex Y23 profile 72 h following digital penetration. Based on this result, it is likely that either full or partial Yfiler Plus profiles will be achieved beyond this 44 h timeframe and warrants further investigation to determine the maximum detection timeframes with this system.

3.4. Effect of type of sexual penetration on Yfiler Plus results

The number of alleles recovered using Yfiler Plus was compared to the type of alleged sexual penetration. The results are presented in Fig. 5. Many highly informative Yfiler Plus profiles were obtained across the different penetration categories and there was no significant difference in the overall recovery of alleles between samples taken for alleged penile penetration, digital penetration, combined digital and penile penetration, or where the type of penetration was unknown ($p = .81$, Kruskal-Wallis test). There were insufficient samples for comparison purposes in relation to oral penetration. The number of alleles recovered ranged from 0 to 33 (average 5.9) for penile penetration ($n = 54$), 0–28 (average 4.5) for digital penetration ($n = 29$), 0–27 (average 7.2) for combined penile and digital ($n = 11$), and 0–30 (average 7.1) where the type of penetration was unknown ($n = 9$). For the one oral swab, 8 alleles were detected.

It could be hypothesised that penile penetration may result in the deposition of more male epithelial cells than digital penetration due to a larger area of contact and the possibility of more friction against the

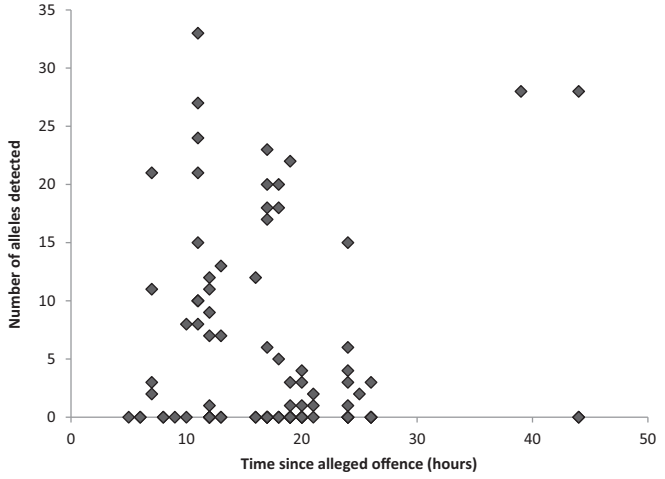

Fig. 4. Number of Yfiler Plus alleles detected versus the time elapsed since the alleged offence for 97 casework samples where time since offence data was available from the medical notes. Least squares regression analysis gave an $R^2 = 0.0012$.

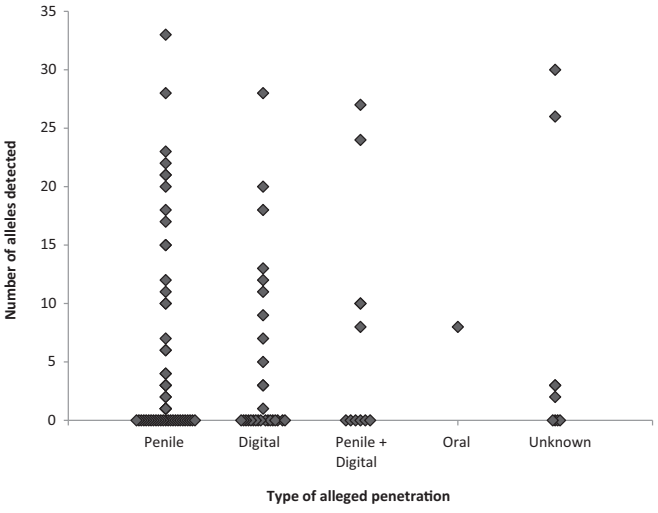

Fig. 5. Number of Yfiler Plus alleles detected versus the type of alleged penetration. The type of alleged penetration was determined from the medical notes.

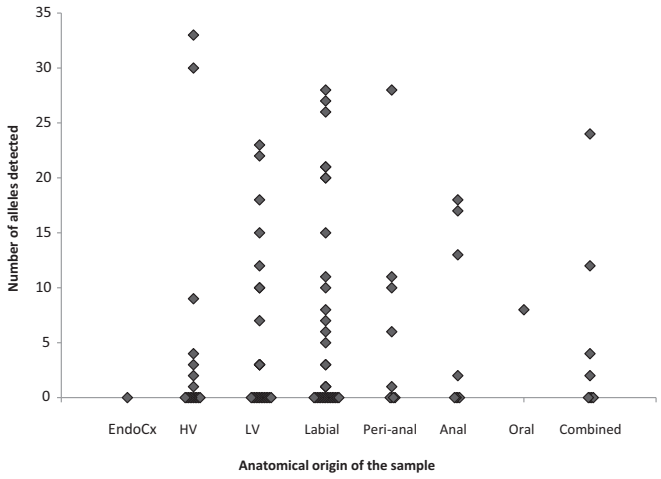

Fig. 6. Number of Yfiler Plus alleles detected versus the anatomical origin of the sample. The anatomical origin was determined from the medical notes. EndoCx = endocervical, HV = high vaginal, LV = low vaginal. Combined swabs were two or more swabs taken from different anatomical origins.

vaginal wall. However, our results indicate that one particular mode of penetration (or a combination of modes) does not result in the deposition of more male DNA than any other. This is most likely due to a range of other variables which would affect the amount of recovered male DNA such as the shedder status of the donor at the time of the offence, the length and force of contact, the vaginal environment, the activities of the complainant post-offence, and the efficacy of sampling.

3.5. Effect of anatomical origin of the sample on Yfiler Plus results

The samples were categorised as endocervical, high vaginal, low vaginal, labial, peri-anal, anal and oral as per the medical notes in the casefile. The number of alleles recovered using Yfiler Plus was compared to the anatomical origin of the sample and the results are presented in Fig. 6. There was no significant difference in the overall recovery of alleles between high vaginal, low vaginal, labial, peri-anal, anal and combined swabs (p = .95, Kruskall-Wallis test). There were insufficient oral and endocervical samples for comparison purposes. The number of alleles recovered ranged from 0 to 33 (average 4.8) for high vaginal samples (n = 17), 0–23 (average 5.2) for low vaginal samples (n = 25), 0–28 (average 6.7) for labial samples (n = 35), 0–28 (average 6.2) for peri-anal samples (n = 9), 0–18 (average 6.3) for anal samples (n = 8) and 0–24 (average 5.3) for combined samples (n = 8). Eight alleles were detected in the oral swab sample but no alleles were detected in the endocervical sample (n = 1 for each).

A study by McDonald et al. [2] suggested that combining samples from multiple locations may improve the recovery of male DNA in no semen cases. However, there was no evidence to support this in our study although the number of combined samples was small (n = 8). Previous studies have shown that persistence of spermatozoa in the anal canal is much shorter than in the vaginal canal and is unlikely to be detected beyond 24 h [11]. In this study, quite informative profiles were obtained from three of the anal swabs despite them being collected at either 13 or 17 h after the offence. As there were no samples tested beyond this 17 h time point, it is difficult to conclude whether epithelial cells may also be lost from the anal cavity at a faster rate than the vaginal cavity. Whilst the loss of male DNA from an external location such as the labia is more probable than an internal cavity such as the vagina for reasons such as washing, urination and contact with clothing, our study showed no significant difference in the likelihood of obtaining a profile from an internal sample versus an external sample. The reason for this is unclear but, as discussed in earlier sections, it appears that the probability of recovering male DNA is dependent on many variable and compounding factors.

4. Conclusions

This study has demonstrated that Yfiler Plus is extremely useful for the detection of low level male DNA encountered following digital penetration and/or penile penetration without ejaculation and has superior performance to its predecessor, Yfiler. The increased sensitivity of Yfiler Plus translated into DNA profiles being obtained in 50% of all samples tested as compared to 25% for Yfiler. It is not known whether the inability to detect male DNA in the remaining 50% of samples is due to the complete absence of male DNA or it being below the limit of detection of the Yfiler Plus kit. As Y-STR markers are linked on the Y chromosome, the discrimination power of a Y-STR profile is relatively poor in comparison to an autosomal profile. Our study has shown that Yfiler Plus generates a significantly higher number of informative alleles than Yfiler and thus will enable much better evidential weight to be provided in the event of a profile match. In addition, Yfiler Plus was able to detect more contributors to male DNA mixtures than Yfiler.

Most forensic DNA laboratories performing Y-STR analysis will typically attempt to quantify male DNA in the sample prior to proceeding to PCR. Our results indicate that the Thermofisher Quantifiler Trio PCR kit, which concomitantly measures male and total human DNA in a sample, has a limit of detection comparable to Yfiler Plus. Increasing amounts of male DNA in a sample were shown to correlate to an increase in the number of Yfiler Plus alleles detected. Therefore, laboratories can use the Quantifiler Trio Y target result to effectively assess the likelihood of obtaining a probative male profile from a sample. However, a sample with undetectable male DNA did produce a useful Yfiler Plus profile in some instances so proceeding to PCR is advisable if no other evidence exists.

Full Yfiler Plus profiles were generated up to 44 h post offence. Samples beyond this timeframe were not included in this study but it is likely that full or partial profiles may be obtained beyond this. The ability to generate an informative Yfiler Plus profile was not related to the time elapsed since the offence, the type of penetration, or the anatomical origin of the sample. This indicates that the deposition and persistence of male epithelial cells does not necessarily follow a similar pattern to the deposition and persistence of semen and spermatozoa. The reasons for this are unknown and certainly warrant further study. However, factors such as the shedder status of the donor, the length and force of contact, the amount of friction, and the efficacies of sampling and DNA extraction may certainly be related to profiling success.

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Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2019.05.001.

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