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Y-STR analysis of digital and/or penile penetration cases with no detected spermatozoa

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ABSTRACT

This forensic casework trial involved Yfiler® testing samples from 47 digital and/or penile penetration cases where the medical examination had occurred within 48 h of the alleged incident and no spermatozoa had been detected following Sperm Elution©. 30% of these cases yielded at least one Y-STR profile comprising three or more alleles per profile and 21% yielded at least one Y-STR profile of ten or more alleles per profile. This trial further investigated the persistence of male DNA in different case types, the location of samples submitted for testing and whether samples from different locations benefit from being combined prior to testing. The data supports the use of Y-STR profiling to provide scientific evidence to investigate whether the alleged sexual activity had occurred as well as to obtain probative evidence in spermatozoa negative penetration cases.

1. Introduction

Cellmark Forensic Services is contracted to undertake forensic analysis on samples submitted by Police Forces in England and Wales and is a supplier of autosomal DNA profiles to the UK National DNA Database. In 2009 a two phase recovery method, Sperm Elution© [1], was introduced at Cellmark Forensic Services (CFS) for the routine extraction of sexual assault samples. This method was shown to have significantly improved sperm recovery from swabs and fabric compared with previous water-based elution methods as well as a greater separation of male and female DNA when used in conjunction with a differential extraction technique. The efficiency of spermatozoa recovery from swabs more than doubled to approximately 71%. Sperm Elution has since been modified resulting in even better separation of male and female DNA as well as a quicker process. CFS performs over 4000 intimate swab Sperm Elution extractions each year. Sperm Elution with differential extraction is capable of detecting spermatozoa and of producing autosomal male DNA profiles up to and including seven days after sexual intercourse with ejaculation. Seven days is the current recommended time limit for taking intimate swabs in medical examinations following an allegation of penile penetration in England and Wales. At the time of this study, 12 h was the recommended time limit for taking intimate samples in allegations of digital penetration but this has been increased to 48 h since the completion of this study (FFLM recommendations [2]).

Despite this increased ability to detect and profile spermatozoa in sexual offence cases, in the past year a significant number of sexual offence allegations examined at CFS were recorded as spermatozoa negative. The lack of detectable spermatozoa could be due to many reasons including a false allegation, an azoospermic or vasectomized perpetrator and no ejaculation.

Research published in 2002 [3] showed that male epithelial cells can transfer into the vagina during vaginal intercourse without ejaculation and male DNA can subsequently be detected. The mechanism was thought to be cells sloughing off the penis during the act of intercourse or due to the presence of male saliva. Semen from a vasectomized or azoospermic perpetrator would still be expected to contain non-sperm male cells including epithelial cells from the ejaculatory duct and urethra [4]. Further research has shown that male epithelial cells can be detected in vaginal samples taken following sexual intercourse with a vasectomized man [5]. It was postulated that male cells could also transfer in the act of digital penetration.

Y-STR typing targets the male DNA present on the Y chromosome allowing production of a Y-STR profile even in the presence of seemingly overwhelming amounts of female DNA. In 1997, Prinz et al. [6] stated Y-STR typing in a male/female cell mixture has a sensitivity in cell ratios of up to 1:2000, whereas, the limit of male DNA detection is 1:50 for autosomal DNA. With the

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recent release of new Y-STR kits, the sensitivity and performance of Y-STR testing has improved over that offered by Yfiler®. Y-STR typing could therefore be expected to be effective on female intimate swabs in situations where penile or digital penetration has occurred and no spermatozoa have been detected. Y-STR profiles cannot currently be loaded to the UK National DNA Database and matching Y-STR profiles provide lower evidential value than autosomal DNA testing. However, Y-STR profiling can provide evidence to investigate whether an incident has occurred as well as produce evidence of probative value where autosomal DNA analysis has not, and/or to link cases.

The ability to obtain Y-STR profiles from intimate swabs and other samples in sexual assault allegations where no spermatozoa have been detected has been previously reported [7–11]. Sibille et al. [7] published in 2002, detected male DNA in spermatozoa negative cases of oral, anal and vaginal penetration beyond 48 h and up to 8 days post incident postulating that the sensitivity of detection using Y-STR amplification could be higher than cytology. However with improvements in spermatozoa recovery, DNA extraction methods and Y-STR profiling kits it was felt that a new study was needed to investigate current success rates. In a recent Australian retrospective study which used Yfiler® testing of vulval swabs in digital and penile penetration allegations where no spermatozoa had been detected [11], it was found that approximately one third of vulval swabs produced a positive Yfiler® result (3–17 alleles). The work concluded that in cases where there is an allegation of digital penetration the vulval swab should be taken within 12 h of the assault. Whilst approximately 40% of vulval samples taken within 12 h of an allegation of penile penetration gave a Y-STR profile of six or more alleles only one of three samples tested between 12 and 24 h was similarly successful.

This casework trial was designed to determine the success rate of Yfiler® testing in allegations of digital and/or penile penetration where no spermatozoa had been detected following Sperm Elution and to investigate the length of time post incident that a Y-STR profile could be obtained in such cases. The number of alleles detected was recorded and two categories of positive Y-STR result were noted: those containing three to nine alleles and those containing $\geq$ 10 alleles. Further, this study was also designed to determine whether quantification using the Investigator Quantiplex® HYres kit from Qiagen could be reliably used as a screening tool in determining which samples will yield a Y-STR profile. A 48 h time limit was chosen for this casework trial given the previous results of the Australian study.

2. Method

Twenty-six police forces in England and Wales agreed to have their digital and/or penile penetration cases considered for inclusion in this Y-STR casework trial. As routine, the intimate swabs taken during the complainant's medical examination were extracted using Sperm Elution [1], which elutes a cellular rich epithelial pellet prior to re-extracting the sample to create the spermatozoa rich pellet. 10% of each resulting pellet was examined microscopically for the presence of spermatozoa.

If no spermatozoa were detected then the case was reviewed for possible entry into the Y-STR casework trial by determining whether the case met the following criteria:

- Female complainant.
- No spermatozoa detected.
- The complainant had no previous sexual intercourse in the 10 days prior to the alleged incident.
- The complainant's medical examination was within 48 h of the alleged incident.

The majority of swabs submitted for the trial were vulva and low vaginal swabs. A few swabs from other intimate locations were also submitted including high vaginal swabs and, in the instances where the complainant lacked knowledge of what had occurred, perianal swabs were also included.

The epithelial pellets from accepted cases were then submitted for DNA testing. Prior to submission, the scientist determined whether the epithelial pellets from different locations would be combined for DNA analysis or kept separate based on the case circumstances, for example, time from the alleged incident to the medical examination and the specific alleged acts.

The epithelial fraction of the appropriate samples (or combination of samples) were submitted for DNA analysis on the EZ1 biorobot (Qiagen) using the EZ1 DNA Investigator kit with the addition of proteinase K and DTT (1 M). The resulting DNA extracts were quantified using Quantifiler® (Life technologies) and then analyzed using 28-cycle SGMPlus® (Life Technologies) at 1 ng (or at 10 μl if the sample quantified at <0.1 ng/μl) following manufacturer's instructions at 25 μl reaction volume. Electrophoresis took place on the 3130xl (Applied Biosystems) using 1 μl PCR product to 9 μl Hi-Di™ formamide/GeneScan™ 400HD Rox with run conditions of 3 kV for 10 s. GeneMapper ID v3.2.1 was used to analyze the resulting DNA profiles. The SGMPlus® results were subsequently reviewed and the DNA extract then submitted for Y-STR analysis using AmpFLSTR® Yfiler® (Life Technologies). If sufficient DNA extract remained, it was subjected to quantification using the Investigator Quantiplex HYres kit (Qiagen) to determine whether any male DNA was detectable at quantification.

3. Results

A total of 47 cases comprising 75 swabs were accepted into the Y-STR casework trial and subjected to DNA analysis (SGMPlus® and Yfiler®). These cases comprised 25 allegations of penile penetration (40 sets of swabs), 11 allegations of digital penetration (14 sets of swabs), 10 allegations which involved both digital and penile penetration (19 sets of swabs) and one case where the form of penetration (digital and/or penile) was unknown (2 sets of swabs). A 'set' of swabs comprised one or more swabs from the same location, for example two low vaginal swabs or one vulval swab. The number of locations sampled could vary from case to case. Where more than one swab was provided from one location they were combined for the purposes of examination and DNA testing. A sample refers to an extract made from one or more swabs from the same location.

3.1. Presence of detectable male DNA in SGMPlus® profiles

Only two of the eleven samples tested which went on to yield a Y-STR profile with $\geq$10 alleles generated an SGMPlus® profile with a suggestion of DNA present from anyone other than the complainant. The additional DNA in these two samples was at trace level and not suitable for meaningful comparison with reference samples. Autosomal profiles obtained from the samples yielding Y-STR profiles containing between three and nine alleles only indicated the presence of DNA matching the complainant.

3.2. Sensitivity of Quantiplex vs Yfiler®

11 out of 75 samples tested generated Y-STR profiles with $\geq$10 alleles. Of these 11 samples, four showed the presence of male DNA present at dual quantification using Quantiplex. All four were at low level: two at 0.0045 ng/μl, one at 0.001 ng/μl and one at 0.0001 ng/μl. No male DNA was detected by dual quantification using Quantiplex in the other samples that produced Y-STR profiles