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Evaluation of usefulness of further Y-STR analysis in sexual assault cases on PSA positive samples resulting in female autosomal STR profiling

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ABSTRACT

Samples from male to female sexual assault cases that are positive for the presumptive prostate specific antigen (PSA) test often do not result in a male autosomal STR profile. Due to highly unequal proportions of female and male DNA in typical sexual assault samples, routine autosomal STR analysis often fails to detect the DNA of the assailant, even after differential extraction of the samples. Previous studies have already shown the value of Y-STR analysis in such cases [1]. In Belgium, forensic DNA laboratories are only allowed to perform Y-STR profiling on sexual assault samples by a specific requisition, after routine autosomal STR analysis has been performed. However, a request for additional Y-STR analysis is rather exceptional.

In this study, we evaluated the usefulness of further Y-STR analysis. For 100 PSA positive rinses and swabs from male to female sexual assault cases resulting in female autosomal STR profiling, 7% resulted in a full or partial Y-STR profile useful for comparison, using the 23-loci Y-STR PowerPlex Y23 System (Promega). The success rate raised to 12.5% with a higher DNA input in the PCR mix. In conclusion, these results support the usefulness of performing Y-STRs analysis on the sperm DNA extracts to identify the alleged assailant in sexual assault cases.

1. Introduction

Sexual violence is a common crime worldwide. In forensic laboratories, sexual assault samples are among the most difficult type of samples due to several reasons such as the very small amount of male DNA compared to the relatively high amount of female DNA, the extended time interval between the crime and sample collection and aged samples where sperm cells may be degraded.

In Belgium, sexual assault cases are often closed after classical autosomal STR analysis reveals no DNA profile useful for identification of the suspect. Here, we performed a study to assess the success rate of Y-STR analysis [1] in cases where autosomal STR analysis was not successful in identifying the offender.

2. Material and methods

2.1. Samples

100 samples (vaginal and/or anal rinses and swabs from sexual assault evidence kits collected between 2003 and 2015) from 65 male to female sexual assault cases which were PSA positive after the presumptive test with the PSA-CHECK-1 test (Veda Lab), which had resulted in female autosomal STR profiling, were selected. In-house validation of the PSA-CHECK-1 test showed a detection limit of 2 ng/ml PSA. All samples were stored at −20 °C and kept frozen until examination.

2.2. Differential extraction

For the rinses, cells were pelleted from a 1.5 ml aliquot by centrifugation. The cell pellet was resuspended in 500 µl buffer A (10 mM EDTA (pH 8.0, 0.2 M NaCl, 10 mM Tris HCl (pH 8.0)) with 30 µl 20% SDS and 15 µl proteinase K (10 mg/ml, Qiagen). For the swabs, the whole swab was cut into small pieces and incubated in the lysis buffer as above. Incubation was performed at 56 °C for 2 h. Next, the lysate was cleared from the swab remnants with DNA IQ Spin Baskets (Promega). The lysates from the rinses and swabs were again pelleted. The non-sperm DNA-containing supernatant was transferred to a new tube. The pellet containing sperm cells was washed three times in buffer A. Sperm cells were lysed in 500 µl buffer A with 55 µl 20% SDS, 25 µl 1 M DTT and 20 µl proteinase K (10 mg/ml) by overnight incubation at 37 °C. Both the non-sperm DNA and the sperm DNA extracts were

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purified with the QIAamp DNA Mini Kit (Qiagen) as described by the manufacturer. DNA was eluted in 150 µl buffer AE (Qiagen).

2.3. Quantification and Y-STR analysis

The amount of total human DNA of the sperm DNA extracts was quantified using the Quantifiler Human Kit (Applied Biosystems) on ABI Prism 7000 Sequence Detection System (from January 2003 till October 2014) or AB 7500 Real-Time PCR System (from October 2014) following manufacturer's recommendations. 23 Y-STR loci were amplified using the PowerPlex Y23 System (Promega). The samples were amplified on a GeneAmp PCR System 9700 (Applied Biosystems). Amplification and thermal cycling parameters were according to the manufacturer's instructions. The PCR fragments were separated by capillary electrophoresis (CE) on an 3130*xl* Genetic Analyzer (Applied Biosystems). DNA profiles were analysed using GeneMapper ID-X software (Applied Biosystems).

3. Results and discussion

The first experiment, containing the analysis of a set of 50 samples, was performed with amplification parameters and thermal cycling conditions according to the manufacturer's protocol, using 2.5 µl DNA extract in the amplification mix. One microliter of the amplified products in a mixture of 9.5 µl Hi-Di Formamide (ThermoFisher) and 0.5 µl CC5 Internal Lane Standard 500 Y23 (Promega) was loaded onto the CE instrument.

Only 3 out of 50 samples resulted in a partial Y-STR profile. All other samples resulted in a negative Y-STR profile. Enhancing the amount of amplification product used for fragment analysis from 1 µl to 3 µl gave no better results. In an attempt to improve the success rate of Y-STR profiling, the DNA input for PCR amplification was adjusted, this by using the maximum amount of 17.5 µl DNA extract in the amplification mix. For economic reasons, this adjustment was in first instance only performed on eight of the 50 DNA extracts including the 3 DNA extracts resulting in a partial profile. Results showed that from the three previous partial Y-STR profiles, two profiles became complete after the adjustment of DNA input was made. The third profile stayed partial. The other five DNA extracts which resulted first in negative profiles, stayed negative.

In a second experiment, another 50 DNA samples were analysed with the maximum amount of 17.5 µl DNA in the amplification mix as described above, to reproduce previous improvements in Y-STR profiling. In 36 of the 50 samples no Y-STR DNA profile was obtained. In four of the samples a complete Y-STR profile was generated. In ten of the samples a partial Y-STR profile could be obtained, of which one was still useful for comparison.

Evaluating the results of the two experiments, six complete and one partial Y-STR profiles useful for comparison (7%) out of 100 PSA positive samples resulting in a female autosomal STR profile, could be generated using the Y-STR multiplex PowerPlex Y23 System. If only the results of a higher DNA input in the PCR mix were taken into account, the success rate raised to 12.5% (7/56 samples).

4. Conclusion

The results of this study support the usefulness of performing Y-STRs analysis in sexual assault cases which previously resulted in female autosomal STR results, although presumptive testing for sperm was positive. Also in sexual assault cases without evidence for the presence of sperm, Y-STR analysis is useful to detect DNA of the male offender. With the use of quantification kits such as Quantifiler Trio (Applied Biosystems) that measure simultaneously the total human and human male DNA concentration the DNA workflow can be streamlined and the success rate in sexual assault cases will increase even more.

Declaration of Competing Interest

The authors declare no conflict of interest.

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