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CASE STUDY

Modified Differential Extraction Protocol for the Identification of Suspect: A Case of Sexual Assault

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Abstract:

DNA fingerprinting is a standard recognized technology for individualization of suspect in criminal justice system. The identification of male contributors from high female and low male ratios through different body fluid mix's is still a major issue to address in forensic DNA laboratories. Traditional methods of separation of the male-female fraction are time-consuming and labour intensive and might give variable results. Currently, there remains scope to refine the method of differential extraction for better results. In the present case study, differential extraction was modified and it was noted that total male DNA yield was 1.561 ng/µl and 2.675 ng/µl in the spermic fraction of the vaginal slide and underwear of the victim, respectively. Although the underwear of the suspect contains female DNA in the non-spermic fraction was 7.864 ng/µl from the underwear of the suspect. Therefore, complete autosomal STR DNA profile of suspect was obtained from the victim vaginal slide and underwear due to significantly reducing the excessive quantity of female DNA using modified differential DNA extraction process. Thus, a modified method is recommended for a better male autosomal STR DNA profile in sexual assault cases.

Keywords: Sexual assault; Differential extraction; Spermic fraction; Non-spermic fraction.

Introduction:

According to the National Crime Record Bureau (NCRB) sexual assault cases increased from 2005 to 2021 in India.[1,2] This data does not involve many sexual assault cases that were not reported or registered.[3] Moreover, there remains a huge backlog of such cases for processing in forensic and crime labs all over the country.[4,5] It is challenging to develop a robust and efficient method for producing full STR profiles of male DNA from a sexual assault sample with an excess of female epithelial cells.[6] Typically, the samples (swab, vaginal smear and clothes of the victim) recovered from sexual assault cases contain huge amounts of female DNA; hence the removal of female DNA from the male portion is very important in order to enhance the recovery of male sperm cells.[7,8] Traditional methods of separation of the male-female fraction are time-consuming and labour intensive and might give variable results.[9,10] Earlier, attempts were made to separate the male and female portions with a high reduction of female DNA carryover in the male DNA portion and minimizing male DNA loss[11,12] Y-STR or autosomal analysis is useful for the detection of the male component in a mixture of male and female DNA. The analysis of autosomal or Y-STR profiling depends on the quantity of DNA in the samples[13,14] Y-STR can confirm the presence of the Y chromosome, while an autosomal STR profile can be used to establish the identity of a male suspect.[15] Basically, the analysis of autosomal or Y-STR DNA depends on the male/female proportion of DNA in the mixed samples of rape cases.[16] Therefore, a comparative study was carried out to evaluate three different buffers with modifications in their incubation times to recover the maximum male DNA percentage.

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Case history and samples: A case was reported in which a 19-years-old victim was sexually assaulted when her parents had gone to work. A person who was living near her barged into her house and asked for some money but the girl denied it and said she did not have any money. The person was aggressive and he dragged the girl forcefully and raped her. During the medico-legal examination of the victim it is a duty of medical practitioner to collect important forensic evidence with standard protocol that may lead to justice.[17] In this case, vaginal slide, underwear and blood sample of the victim were collected by a medical officer. Two samples were collected from the suspect i.e., underwear and a blood sample during the medico legal examination and sent to a forensic science laboratory for investigation. Vaginal slides, underwear and blood of the victim were also collected for the present study in order to confirm the presence of the suspect's DNA and underwear of suspect for the analysis of female DNA. Suspect's blood sample was preferred as a reference sample for matching the suspect's DNA in all samples.

Methodology:

3.1 Direct lysis: Single-step lysis and differential lysis were performed with all three samples, i.e., vaginal slide, underwear of

Table 1. STR Profile of all processed samples with the recovered amount of DNA through a modified differential extraction procedure.

Exhibits	Direct lysis/ Differential lysis	Y-STR DNA Profile	Autosomal STRDNA Profile
Vaginal Slide of Victim	Direct Lysis	Complete Profile	Female Profile
	Spermic Fraction	Complete Profile	Male Profile
	Non-Spermic Fraction	Partial Profile	Female Profile
Underwear of Victim	Direct Lysis	Complete Profile	Female Profile
	Spermic Fraction	Complete Profile	Male Profile
	Non-Spermic Fraction	Partial Profile	Female Profile
Underwear of Suspect	Direct Lysis	Not Done	Mixed Profile
	Spermic Fraction	Not Done	Male Profile
	Non-Spermic Fraction	Not Done	Female Profile

the victim and underwear of the suspect. In direct lysis, suspect stains were taken in a 2ml microcentrifuge tube. Lysis was performed in 500 µl of: *buffer C (Modified Differential Buffer), 50 µl of 20% SDS, 20 µl proteinase K of 20 mg/µl and 20 µl DTT of 1M. All samples were Incubated at 56°C for 1 hour.

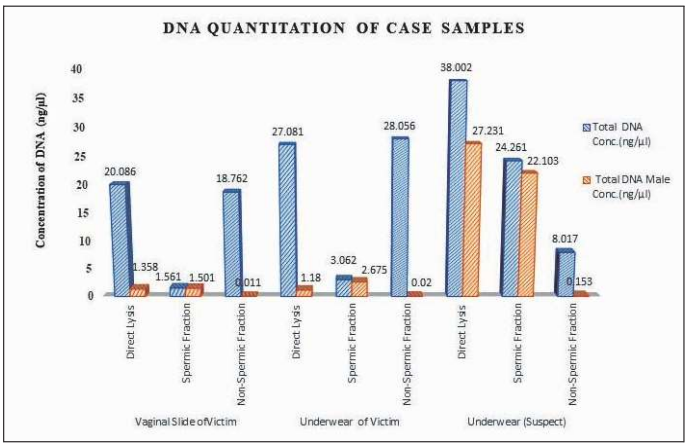
*Buffer composition will be disclosed later, as some studies are pending with this buffer.

3.2 Differential Lysis: Differential extraction was performed for the separation of male and female from the vaginal slide and clothing of the victim. Stains of the suspect were taken in a 2ml microcentrifuge tube. Preliminary lysis was performed in 500µl of buffer C (Modified Differential Buffer), 50µl of 20% SDS and 20 µl proteinase K of 20mg/µl. Both samples were incubated at 56°C for 30 min. Subsequently, samples were placed in spin basket for centrifugation at 7500 rpm for 3 mins. Spin baskets were then discarded and the samples were centrifuged again for male pellets. The remaining portion called female supernatant, was transferred into a new 1.5 ml microcentrifuge tube. The male portion was further lysed in a 400µl C buffer solution, 50µl of 20% SDS, 20 µl proteinase k of 20 mg/ µl and 20µl 1M DTT which was incubated at 56 °C for 30 min. DNA purification was done using automated DNA extraction system with Profiler™ Forensic DNA Extraction Kit as per manufactured protocol (Thermo Fisher, USA).

3.3 Quantification and Amplification: Isolated DNA was quantited by 7500 Real time PCR system (Thermo Fisher, USA) with the Promega Power Quant System kit as per manufacturer.[18] Autosomal STR and Y-STR marker amplification was performed using PowerPlex Fusion 6c multiplex system (Promega, USA) and PowerPlex Y23 multiplex system (Promega, USA), respectively as per manufacturing protocol. Amplification was done on VeritiTM 96-Well Fast Thermal Cycler (Applied BiosystemsTM).[19,20]

3.4 Genotyping: Amplified DNA fragment were analyzed on the Genetic Analyzer 3500 XL (Applied BiosystemsTM) using size standards and an allelic ladder provided by the manufacturer with the respective multiplex systems.[21,22] Data was analyzed through gene mapper software IDX 1.5 (Applied Biosystems TM).

3.5 Ethical clearance: This study ethical clearance was approved by the Institutional ethical clearance committee of Amity University, Haryana, vide latter No. IEC-AIB/AUH/2022-6, Date: 6th June, 2022.



Results: The selectivecase was examined through the standard differential extraction protocol with some modification. The RT-PCR data was obtained as described in Figure 1.

In the vaginal slide of the victim, the total DNA was found 20.086 ng/µl from which male DNA was only 1.358 ng/µl through the direct lysis process, whereas in the modified differential lysis procedure, the spermic fraction contains 1.561ng/µl total DNA from which the male DNA was found 1.501 ng/µl. Non-spermic fraction contains 18.762ng DNA and male DNA quantity was 0.011ng/µl.

In sample 2, underwear of victim the total DNA was found 27.081 ng/µl from which male DNA was only 1.18 ng/µl through the direct lysis process, whereas in the modified differential lysis procedure,the spermic fraction contains 3.062 ng/µl total DNA from which the male DNA was found 2.675 ng. Non-spermic fraction contains 28.056ng/µl DNA and male DNA concentration was 0.02ng/µl.

In sample 3, underwear of the suspect the total DNA was found 38.002 ng/µl from which male DNA was only 27.231 ng through the direct lysis process, whereas in the modified differential lysis procedure,the spermic fraction contains 24.261 ng total DNA from which the male DNA was found 22.103 ng. Non-spermic fraction contains 8.017ng DNA and male DNA content was 0.153ng.

STR profiles were obtained through all exhibits described in Table1.

Discussion and Conclusion:

The recovery of total DNA and male DNA concentrations with direct lysis and differential lysis, spermic fraction and non-spermic fraction were checked for quantity with two methods. Consequently, the reference blood sample of the suspect was analyzed to match the sample with the source of victim.

In sample 1,the direct lysis method was applied in the vaginal slide; DNA quantity of the female was found to be higher (18.728ng/µl) as compared to the quantity of male DNA (1.358ng/µl). The male to female DNA ratio was observed 1:13.79. Thus, we obtain a female autosomal STR profile due to the higher carryover of female DNA on male DNA. Consequently, differential extraction method was applied in

another replicate and two fractions were analysed i.e., the spermic fraction (MF) that contains1.501ng/µl male DNAand female DNA was reduced and foundin less quantity (0.06ng/µl). In non-spermic fraction the male loss was very minimal (0.011ng/µl). Due to differential extraction method, we obtained autosomal male DNA profile in the spermic fraction.

In sample 2 (underwear of victim)was processed with direct lysis and the female DNA quantity was found to be high quantity(25.901ng/µl)and the male was found to be low (1.18 ng/µl), where the male to female ratio was 1: 21.95. So, we are not able to obtain a male autosomal STR profile due to the excess amount of female DNA. In differential extraction method, the spermic fraction (MF) contains 2.675 ng/µl male DNA and the non-spermic fraction contains 28.036 ng/µl female DNA. The female carryover was reduced maximum and found to be only 0.387ng/µl in the spermic fraction through the modified differential method with lysis buffer C. Besides, the non-spermic fraction contains minimum loss of male DNA i.e., 0.021ng/µl.

In sample 3 (underwear of suspect) contains 27.231ng/µl male DNA and 10.771ng/µl female DNA through the direct lysis method, whereas in the differential extraction method, the spermic fraction contains 2.158ng/µl female DNA and in the non-spermic fraction the female DNA was 7.864ng/µl. So autosomal male profile was obtained in the spermic fraction and female autosomal profile was obtained in the non-spermic fraction.

The male DNA recovery was maximum with less female DNA carryover in the spermic fraction, whereas the female DNA quantity was higher in the direct lysis process. Although, minimum quantity male DNA loss in the non-spermic fraction. Thus, male autosomal profiling was not obtained through direct lysis of sample due to the high carryover of female DNA. The result of any DNA fingerprinting case depends on the type and condition of the samples which should be collected through standard procedure and proper precautions should be taken by the medicolegal officer. Otherwise, it is hard to get information from samples and the case could be reported as having inconclusive results.

Modified differential extraction was successful applied in sexual assault to identify suspect on victim source and victim DNA profile from successfully obtained from suspect source. Modified differential DNA extraction method will be play significant role in DNA analysis of sexual assault.

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