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Studies of different clue materials for semen detection and DNA fingerprinting in sexual assault cases

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Abstract
DNA fingerprinting is one of the most reliable techniques in forensics with highest possible accuracy and detection limits. The results are crucial to link the suspect to the crime. However it is observed that delay in medical examination prior to the collection of biological samples of victim for DNA fingerprinting reduces the chances of semen, DNA isolation from various exhibits and subsequent detection. A comparative study of analysis of different types of biological samples has been carried out to find the best source for semen detection among the samples collected from the victim. A total of 200 different samples such as swabs from different parts of victim's body, clothing and vaginal wash were examined for the presence of semen based on presumptive tests and microscopic examination followed by DNA fingerprinting. Study shows vaginal wash as best source with respect to uncontaminated semen and quantity of DNA which enable to generate accurate DNA profile.

Keywords: DNA fingerprinting, Sexual assault, clue material, Semen, Vaginal wash

1. Introduction
DNA fingerprinting is the most reliable technique in the present day forensic biological examinations giving results with highest accuracy. In cases involving sexual assault cases, it is essential to isolate and analyze DNA from various samples of victim and the suspect and to compare the DNA fingerprinting profile from these samples. This is done to identify the individuals on the basis of DNA from the spermatozoa present in different samples of victim collected using sexual assault forensic evidence (SAFE) kit ie. evidence such as clothing fibers, hair, saliva, blood, semen or body fluid that may help identify the assailant and provide evidence supporting prosecution in a criminal trial. The delay in medical examination prior to the collection of biological samples of victim for DNA fingerprinting reduces the chances of semen detection on the samples[1]. Further quality & quantity of DNA is also effected by cloth texture [2]. Presence of large amount of epithelial cells of victim in these samples & other environmental factors interferes and complicates the process of analysis[3]. Vaginal wash taken in sterile water or normal saline help to preserves the spermatozoa for long duration. The other samples preserved in non porous container leads to degradation of samples [4].The main objective of this study is to compare the results obtained from different types of biological samples of victim in sexual assault forensic examination kit (**SAFE)** to ascertain the best source for semen detection and DNA quantification, as presence of sufficient amount of DNA is an important aspect in subsequent analysis of DNA fingerprinting [5].

2. Materials and Methods
The exhibits received in the laboratory were stored at 4º C as per standard protocol so that level of degradation was minimised. The detection of spermatozoa affected by the environment factor and cloth texture prior to preserving it at desired temperature [2].

Selection of samples: 200 samples from positive cases of semen in presumptive tests and confirmatory tests [6] (20 from each of the 10 selected samples from SAFE kits) were taken in this study. Location of semen was detected using UV illuminator & the presence of spermatozoa was detected by microscopy using erythrosine fast green differential staining method.

DNA Extraction: DNA extraction from the samples was done using organic DNA extraction method using Phenol and chloroform& purification of extracted DNA was done by using centricon [7].

Quantification of DNA: Concentration of male DNA isolated from each of the sample was measured by quantification method by using Quantifiler Duo Kit on the Real-Time PCR instrument[8-9].

3. Results & Discussion

Amounts of Male DNA (ng/µl) isolated from various samples of victim using RT–PCR are given vide table-1 and relative percentage of male DNA from various samples studied depicted vide chart-1.

Based on concentration of male DNA isolated from various samples (20 samples of each type) following observations were made.

Washing from vagina:18 samples showed positive results with total combined concentration of 326.5 ng while remaining 2 samples showed nil concentration.

Vaginal swabs & smears: 17 samples gave total combined concentration of 231 ng while 3 samples gave no results.

Inner clothing: 18 gave total combined conc. of 220ng while remaining 2 showed no result.

Cervical mucus collection: 18 samples gave total conc. of 63.9 ng while 2 samples gave no results.

Rectal swabs & smear: 9 samples gave positive results with a total combined concentration of 16 ng while remaining 11 samples showed no results.

Outer clothing:8 samples gave positive results with a total combined concentration of 225 ng and 12 samples showed no results.

Culture, Oral & Body fluid swabs: Only 1 sample from each of the exhibits gave positive result with concentration of 1, 1.3 & 1.46 respectively and remaining 19 samples gave no results.

Pubic Hair: Only 2 samples gave positive results with a total combined concentration of 2.28 ng and 18 samples showed no results.

Perusal of results shows that vaginal wash was found to be the best source for DNA isolation and quantification in sexual assault cases. Further, it is relatively free from contamination from other environmental factors and microbial DNA as it is taken in a closed system in liquid state which is crucial for generating accurate DNA profile. Vaginal swabs, inner clothing & outer clothing were also found to be good sources for spermatozoa DNA isolation if timely medical examination is done. However, these samples were often found to be contaminated due to exposure to environmental factors, cloth texture and inhibition due to dyes. Culture swabs, oral swabs, pubic hair and body fluid collection were not considered as good sources due to absence of semen in most of the samples which may be attributed to their location which reduces the chances of semen deposition.

Table1: Concentration of Male DNA (in ng/ µl) isolated from various samples of victim

Sample(s))	Vaginal Wash	Vaginal swabs & smear	Inner clothing	Cervical mucouscoolection	Rectal swabs * smear	Outer clothing	Culture Swabs	Pubic hair	Oral swabs	Body fluid swab
1	37.17	12.9	12.1	0.11	5.02	2.05	0	0	0	0
2	13.28	3.02	7.98	6.08	0.26	0	0	0	0	0
3	0.97	7.96	22.1	0.5	0	18.8	0	0	0	0
4	34.58	18.4	7.59	1.14	1.14	0.08	1.27	1.2	0	1.45
5	17.56	0.07	0.04	1.12	0.01	0	0	0	0	0
6	15.17	4.78	11.3	2.42	0	194	0	0	0	0
7	67.29	19.4	21.4	0	0	0.07	0	0	1.3	0
8	9.24	5.43	5.52	2.22	1.2	1.02	0	1.06		0
9	13.28	0	12.9	1.22	0	0	0	0	0	0
10	5.45	12	16.5	0.07	0	0	0	0	0	0
11	0	50.3	0	3.59	0	0	0	0	0	0
12	16.28	0	33	15.8	4.23	1.25	0	0	0	0
13	7.92	7.26	3.42	7.32	0	0	0	0	0	0
14	20.75	5.78	0.01	2.06	0	0	0	0	0	0
15	0.05	7.09	4.32	3.29	0	7.35	0	0	0	0
17	17.58	50.3	14.4	0.01	0	0	0	0	0	0
18	46.25	17.4	37.4	15.3	2.98	0	0	0	0	0
19	0	8.78	0	1.72	0.07	0	0	0	0	0
20	3.67	0	10.1	0	1.06	0	0	0	0	0
Total Male DNA (ng/ µl)	326.5	231	220	63.9	16	225	1.27	2.2	1.3	1.46
Average Male DNA (ng/ µl)	16.32	11.5	11	3.20	0.8	11.8	0.063	0.11	0.06	0.073

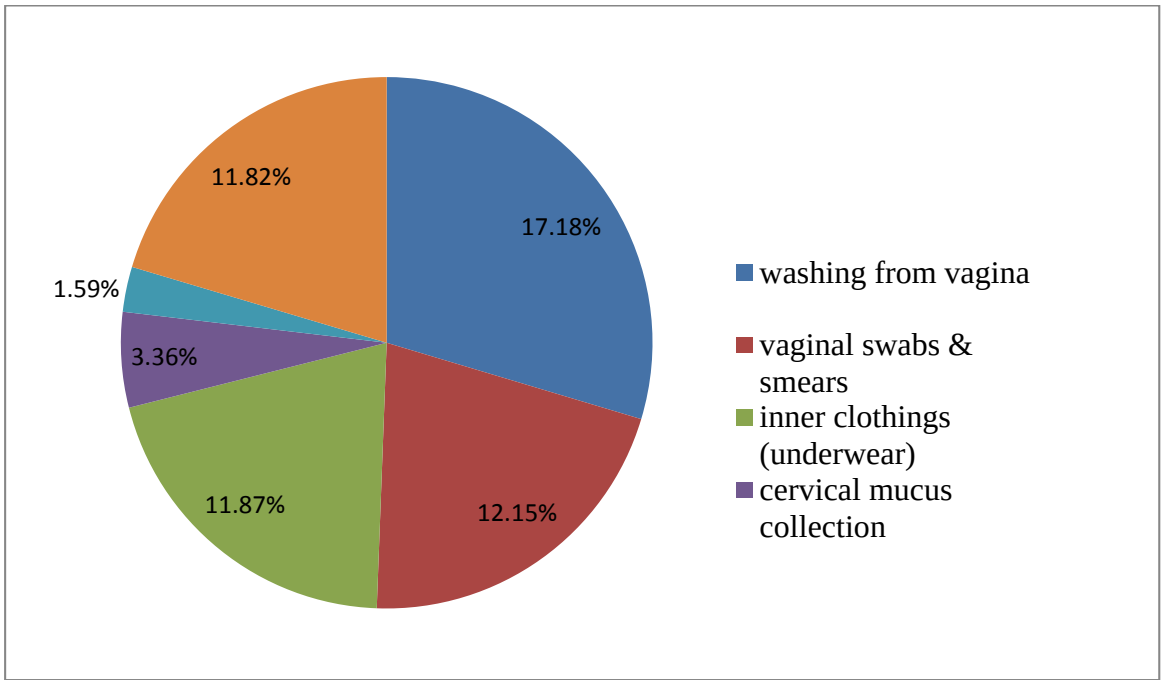

Chart-1: Showing relative percentage of male DNA from various samples

4. Conclusion

From the above results, it was concluded that vaginal wash was found to be among the best source for DNA isolation followed by vaginal swabs & smears, inner clothing, outer clothing, cervical mucus collection, rectal swabs and smears while culture, pubic hair, oral and body fluid swabs were relatively weak sources of DNA. Hence, greater emphasis should be given to washing from vagina, vaginal swabs, inner and outer clothing so as to get reliable results in sexual assault cases.

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