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DNA Profiling from Mixed Samples in Rape Cases

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Abstract: ***DNA analysis has become an indispensable tool in forensic investigations; however, interpretation of mixed DNA samples remains one of the most challenging aspects, particularly in sexual assault cases. Mixed DNA samples contain genetic material from two or more contributors and often produce complex profiles characterized by allele drop in, allele drop out, stutter peaks, and peak imbalance. These complications are further intensified when contributors are biologically related or when samples are degraded due to delayed collection or environmental exposure. The present study aims to improve the interpretation of mixed DNA samples by applying optimized extraction, amplification, and probabilistic interpretation strategies. Enhanced PCR conditions, differential extraction, and Y STR analysis were employed to generate accurate and interpretable profiles from mixed samples. The results demonstrate that the applied methodology improves sensitivity and reliability in identifying contributors in sexual assault cases.***

Keywords: Mixed DNA, STR analysis, Forensic genetics, Sexual assault, DNA profiling

1. Introduction

In forensic DNA laboratories, mixed biological samples are routinely encountered, particularly in cases involving sexual assault. Such mixtures commonly originate from blood and semen, although saliva, hair, and skin cells may also contribute. A mixed DNA profile is generally inferred when more than two alleles are detected at a single locus; however, additional peaks may also arise due to contamination, stutter artifacts, or allelic drop in and drop out.

In many sexual assault cases, a complete DNA profile of the victim is readily obtained, whereas the suspect's profile may be partial or degraded due to prolonged intervals between the crime and laboratory analysis. In gang rape cases, interpretation becomes even more complex as the number of contributors increases, resulting in reduced discriminatory power. Therefore, accurate interpretation of mixed DNA profiles remains one of the most controversial and technically demanding aspects of forensic genetics.

2. Aim

The aim of the present study is to generate interpretable and reliable DNA profiles from mixed samples encountered in forensic casework.

3. Materials and Methods

3.1 Sample Collection

Biological exhibits including cervical swabs, vaginal swabs, smears, clothing, blood samples of the victim, blood stained gauze, nail clippings, and pubic hair of the accused were collected from crime scenes and hospitals. All exhibits were handled with strict contamination control measures during collection and packaging.

3.2 DNA Extraction

DNA was isolated using the organic phenol–chloroform extraction method for bloodstains. Male–female mixtures were processed using differential DNA extraction to separate epithelial and sperm fractions.

3.3 PCR Amplification

Crime scene samples often contain PCR inhibitors that interfere with DNA amplification. To overcome inhibition and low template DNA issues, the following modifications were applied:

- Dilution of genomic DNA to reduce inhibitor concentration.
- Addition of increased DNA polymerase to neutralize inhibitory molecules.
- Use of 30 PCR cycles to enhance amplification of low copy or degraded DNA.

Autosomal STR amplification was performed using the AmpFlSTR Identifiler kit, while Y STR amplification was carried out using the AmpFlSTR Yfiler kit (Applied Biosystems) to specifically identify male contributors in mixed samples.

3.4 Data Analysis

Capillary electrophoresis was performed on an ABI 3500 XL Genetic Analyzer. Data analysis and allele calling were conducted using GeneMapper IDX software in accordance with manufacturer guidelines.

4. Results and Discussion

Careful handling during case opening minimized external contamination. Differential extraction successfully separated male and female fractions. Optimized PCR conditions resulted in improved peak balance and enhanced detection sensitivity.

Electropherogram analysis revealed that mixed profiles typically consisted of balanced peaks corresponding to the victim's profile and imbalanced peaks corresponding to the suspect. Additional peaks observed near true alleles were identified as stutter products and excluded after comparison with the allelic ladder.

Mixed genotypes were interpreted based on the following observations: - Presence of more than two peaks at a locus. - Severe peak height imbalance in heterozygous loci. - Elevated stutter percentages (15–20%).

Quantitative interpretation included peak height comparison, allowing differentiation between heterozygous loci, mixed samples, and stutter artifacts. Loci exhibiting four peaks indicated both contributors were heterozygous, while three, two, or one peak patterns were interpreted based on allele overlap or homozygosity.

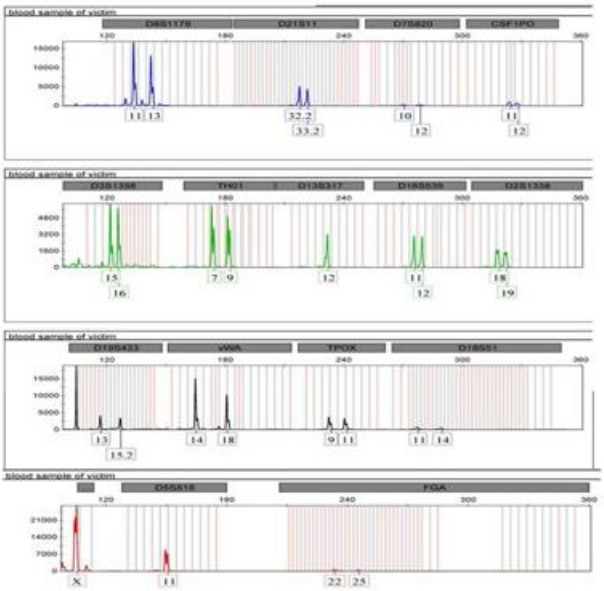

Figure 1: Blood sample of victim

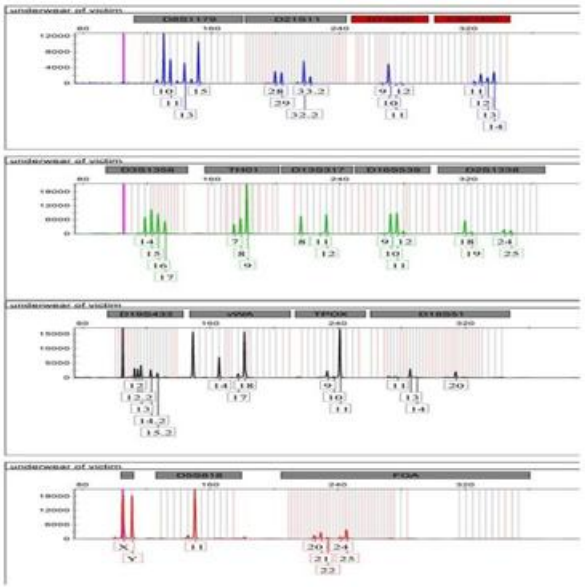

Figure 2: Underwear of victim

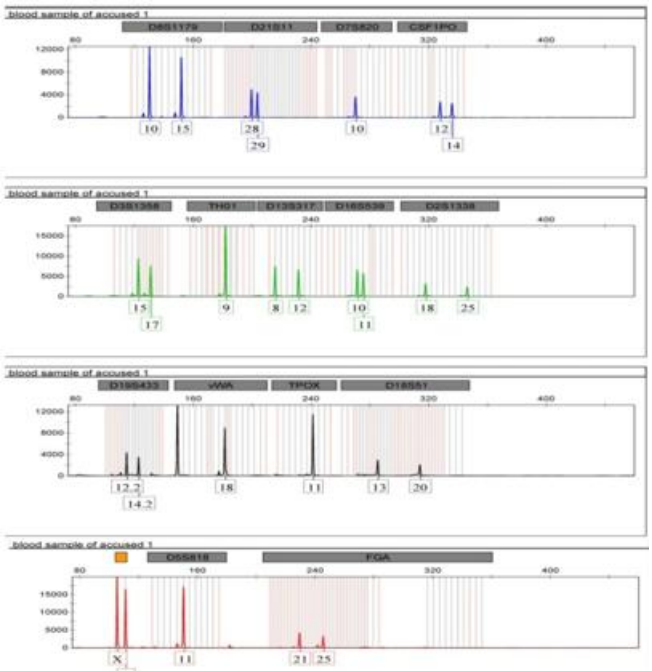

Figure 3: Blood sample of accused 1

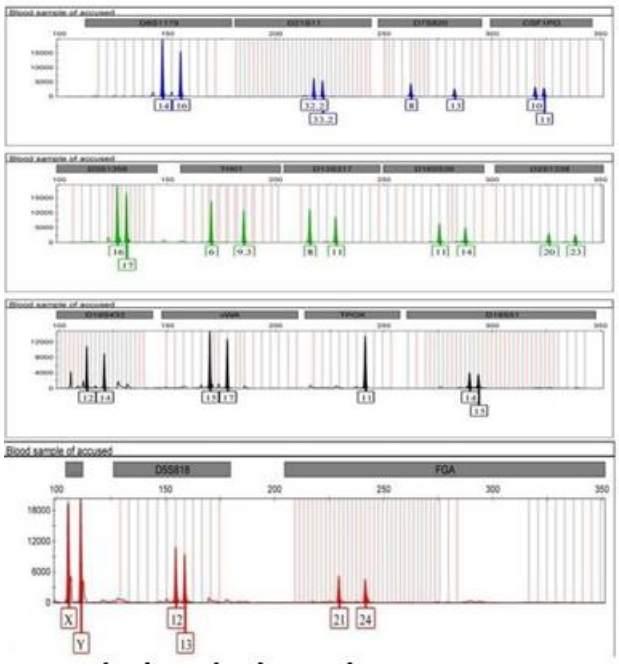

Figure 4: Blood sample of accused 2

5. Conclusion

The mixed autosomal STR profile of the victim's exhibit indicated the presence of two contributors. One set of alleles matched the profile of accused 1, while remaining alleles could not be attributed to accused 2. The presence of additional allelic bands suggested the involvement of more than one perpetrator, which was further confirmed through YSTR analysis, yielding two complete male profiles. The study highlights the importance of optimized laboratory techniques and careful interpretation in resolving complex mixed DNA samples.

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