Forensic Approach Analysis of Multipartner (STR) for Identifying the Suspects in Iraqi Sexual Assault Cases : A Case Study

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ABSTRACT

DNA has become a powerful forensic tool for solving cases such as linking a suspect to a crime scene, resolving biological relationship issues and identifying disaster victims. DNA fingerprinting can be used to help identify individuals by revealing the differences in the DNA sequence among different people. Certain non-coding regions of DNA exhibit significant differences in base pair sequence among individuals. Such regions are called polymorphisms. DNA investigations mainly involve two steps; the obtaining of DNA profiles from biological samples and the interpretation of the evidence given by these DNA profiles. Chromosomal DNA was extracted from semen samples as well as from blood stain more over PCR technique applied to amplify STR loci, while the detection carried out by capillary electrophoresis .Then making the interpretation by matching DNA profiles that obtain from both suspects and victims with the DNA profiles that obtain from the objects in the crime scene. The case involve seven forensic samples, one female victim (A), three suspects (B, C, D) and three clothes (brown underwear, blue jeans and shirt belong to victim A).. DNA extraction from the semen contamination in these samples give DNA fingerprints with a mix profile that's belong to more than one suspect.

Keywords: sexual assault, DNA fingerprinting, mixture interpretations, STR loci, Forensic, likelihood ratio, Case study.

INTRODUCTION

Sexual assault (rape) is a crime of violence against a person's body resulting in a physical trauma, mental anguish, and suffering for victims generating expenses for government intended criminal investigation, medical care, and psychological attention. During the crime scene investigation, the identification and recovery of biological evidence are utmost important, since sometimes these are the only way to prove sexual contact and the perpetrator's identity. The examiner, with the help of specific technologies and techniques, must be able to find evidence that otherwise could go unnoticed (González *et al.*, 2019).

MATERIALS AND METHODS

Subjects

The persons from which the samples are collected that's included:

1-The victims A (sexually abused persons).

2-The suspects B, C and D (perpetrator).

The victims the Medico-Legal Directorate (MLD) deals with includes both deceased and living individuals, sample collection for deceased individuals takes place during autopsy. For living individual it is carried out in the outpatient clinic. Samples include swabs (Buccal, vaginal and rectal), blood on (Flinders technology association) FTA card, clothes, tissue paper (kleenix) and etc.

Sample collection for suspects takes place in the outpatient clinic of Medico-Legal Directorate or in the major hospitals of Baghdad and other governorates; samples include swabs (Buccal, rectal and penis), blood on FTA card and clothes (Kumer *et al.*,2018).

All samples except FTA cards are first sent to the serology laboratory for identification (presumptive and confirmatory tests for blood and semen) and then to the DNA laboratory for advanced processing (DNA extraction, DNA concentration determination by real time PCR, DNA amplification PCR, genetic analysis and DNA profile).The victim A has filed a complaint to the local police authority accusing 4 individuals of sexually assaulting her, 3 of suspects (B, C and D) were taken in to custody while the fourth suspect remained at large, blood samples from the three suspects were obtained on FTA card, together with a blood sample and clothing samples (mentioned in the table 1) from the victim.

Table 1: The forensic samples.

No.	The Forensic Samples	Gender
1.	FTA for victim A	Female
2.	Brown underwear A.	For female
3.	Long blue shirt A.	For female
4.	Blue jeans trousers A.	For female

5.	FTA for suspect B.	Male
6.	FTA for suspect C.	Male
7.	FTA for suspect D.	Male

Sampling

Blood Stain Samples Collection and Direct Amplification of DNA from Storage Card (FTA) Punches for victim A and suspects B, C and D. Presumptive tests for semen by UV LAMP and Confirmatory test by RSID-Semen (Rapid Stain Identification of Human Semen) kit (Forensics,2016) for samples (brown underwear, blue shirt and blue jeans trousers).By the DNA IQ™ Casework Pro Kit for Maxwell® 16 is used with the Maxwell® 16 Instrument configured for low elution volume (LEV) and is specifically designed for optimal DNA extraction from forensic Casework samples (Corporation, 2016). By The PowerQuant® System which is a five dye, four target hydrolysis probes based qPCR multiplex that amplifies multicopy targets to quantify the total human and human male DNA present in a sample. The system also amplifies an additional multicopy target to assess the degree of DNA degradation. Additionally, the PowerQuant® System includes an internal PCR control (IPC) to detect inhibitors in an amplification reaction (Corporation, 2015-2018). Then Polymerase chain reaction, Capillary Electrophoresis and Genetic analysis (pre and post PCR) by The PowerPlex® 21 and Powerplex Fusion Systems using the Applied Biosystems® 3130 XL Genetic Analyzer (Corporation, 2016).The table (2) shows the serological tests and DNA concentration for samples no.2 (brown underwear A), no.3 (long blue shirt A) and no.4 (blue jeans trousers A). And the results are as follows:

Table 2: The results for serological tests and DNA concentration determination.

Sample no.	Serological tests	Real time PCR (DNA concentration)
2	+ ve semen	13.8 ng/ µl
3	+ ve semen	3.4 ng/ µl
4	+ ve semen	1.5 ng/ µl

In this table serological tests showed positive results for all samples (semen), and showed that sample no.2 (brown underwear A) represent the highest DNA concentration (13.8 ng/ µl), while sample no.4 (blue jeans trousers A) represent the lowest DNA concentration (1.5 ng/ µl).

Table 3: PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches using powerple21 and powerplex fusion kits (Corporation, 2016).

Component Powerplex 21	Component Powerplex Fusion	Volume per Reaction
Water, amplification Grade	Water, amplification Grade	15 µl
PowerPlex ®21 5X Master Mix	PowerPlex ®Fusion 5X Master Mix	5.0 µl
PowerPlex® 21 5X Primer Pair Mix	PowerPlex® Fusion 5X Primer Pair Mix	5.0 µl
Total volume	Total volume	25 µl

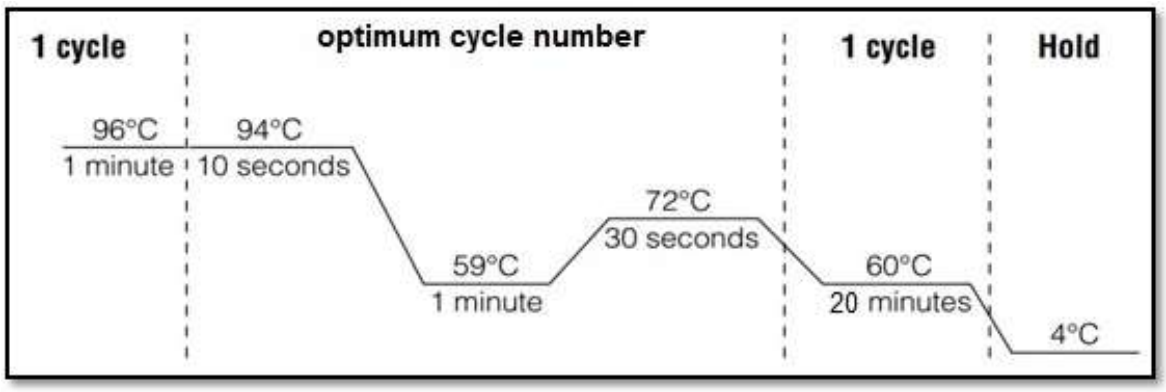

Figure 1: The thermal cycling protocol for the GeneAmp® PCR System 9700 thermal cycler for direct amplification (Corporation, 2016).

Statistical Calculations

By using GeneMapper® *ID-X* Software version 1.5 which is an automated genotyping software solution for all human identification (HID) data analysis needs, including forensic casework, data basing, and paternity testing. Table 4 show statistical calculations parameters (Profile Frequency PF, Random Match Probability RMP, Likelihood Ratio LR and Combined Probability of Inclusion/Exclusion CPI/CPE (Buckleton *et al.,* 2016).

Table 4: statistical calculations

Statistical parameter / Samples	Profile Frequency PF	Random Match Probability RMP	Likelihood Ratio LR	Combined Probability of Inclusion/Exclusion CPI/CPE
FTA victim A	1.5968E-30	1 in 6.2624E29	6.2624E29	----------
FTA suspect B	3.6217E-25	1 in 2.7611E24	2.7611E24	----------
FTA suspect C	2.0491E-28	1 in 4.8802E27	4.8802E27	----------
FTA suspect D	1.3145E-23	1 in 7.6072E22	7.6072E22	----------
Brown underwear A	----------	----------	----------	CPI=1 in 2.8828E1 CPE= 96.5311 % for American
Blue jeans A	9.8208E-26	1 in 1.0183E25	1.0183E25	CPI=1 in 9.0018E0 CPE= 88.8911 % for American
Blue shirt A	1.3145E-23	1 in 7.6072E22	7.6072E22	----------

RESULTS AND DISCUSSIONS

The data analyzed using GeneMapper ®ID software, version 3.2 from applied biosystems, following PowerPlex®21 System and PowerPlex Fusion technical manual to created panels, bins and stutter text files to allow automatic assignment of genotypes. Profiles are considered reportable if results are obtained from a minimum of 18 STR loci and Amelogenin; homozygotes are called with a minimum Relative Fluorescent Unit (RFU) threshold of 300 and heterozygotes are called with a minimum RFU threshold of 50 and each allele should be confirmed by double amplification.

The work conducted by a forensic examiner (i.e., DNA analyst) summarized in to a laboratory report. Which is based on standard operating procedures that must be followed. Prior to release of a lab report, data and conclusions are vetted through an internal review process culminating with a second reviewer and/or the DNA technical leader approving the work (Yang *et al.,* 2019).

Generally, the process of comparing two or more samples is limited to one of three possible outcomes that are submitted in a case report:

1. Inclusion (match): Peaks between the compared STR profiles have the same genotypes and no unexplained differences exist between the samples. Statistical evaluation of the significance of the match is usually cited in the match report. Alternatives for presentation of a match range from statements of identity, to computations of the likelihood ratio for the hypothesis that the defendant is the source, to descriptions of random match probabilities in various populations, to a simple qualitative report of a match with no statistics behind its significance.
2. Exclusion (nonmatch): The genotype comparison shows profile differences that can only be explained by the two samples originating from different sources.
3. Inconclusive: The data does not support a conclusion as to whether the profiles match. This finding might be reported if two analysts remain in disagreement after review and discussion of the data and it is felt that insufficient information exists to support any conclusion (Butler, 2015).

- Typically if 2, 3, or 4 alleles then 2 contributors
- If 5 or 6 alleles per locus then 3 contributors
- If >6 alleles in a single locus, then >4 contributors (Butler *et al.,* 2015).

DNA Fingerprints for References and Mixed Profiles

By using pre and post PCR kits (powerplex21 and powerplex fusion kits), the forensic samples underwent amplification then genetic analysis by capillary electrophoresis using Genetic analyzer 3130 xl and the DNA profiles were obtained by using GeneMapper ID software version 3.2.

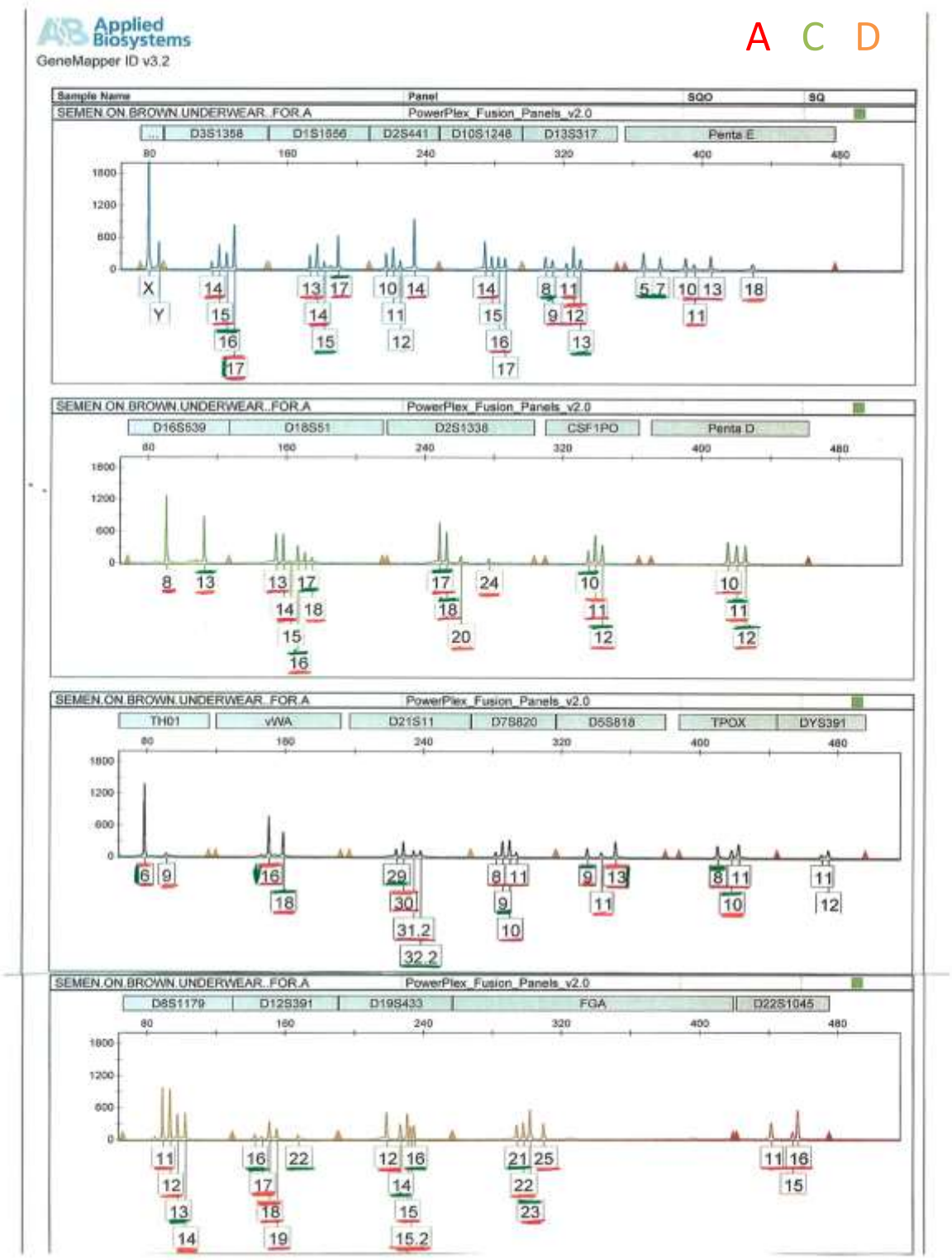

Figure 2: DNA profile for brown underwear A.

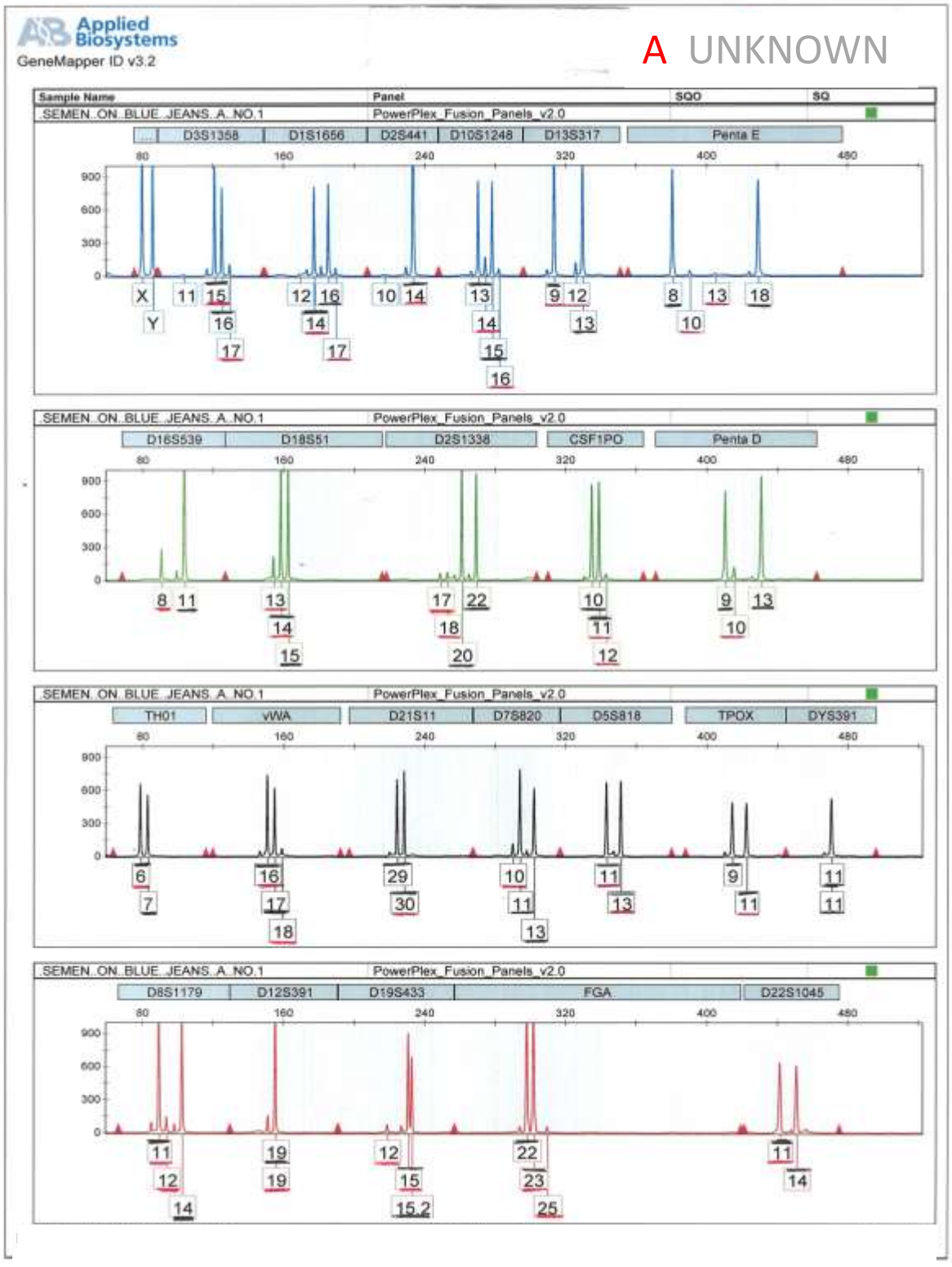

Figure 3: DNA profile for blue jeans A.

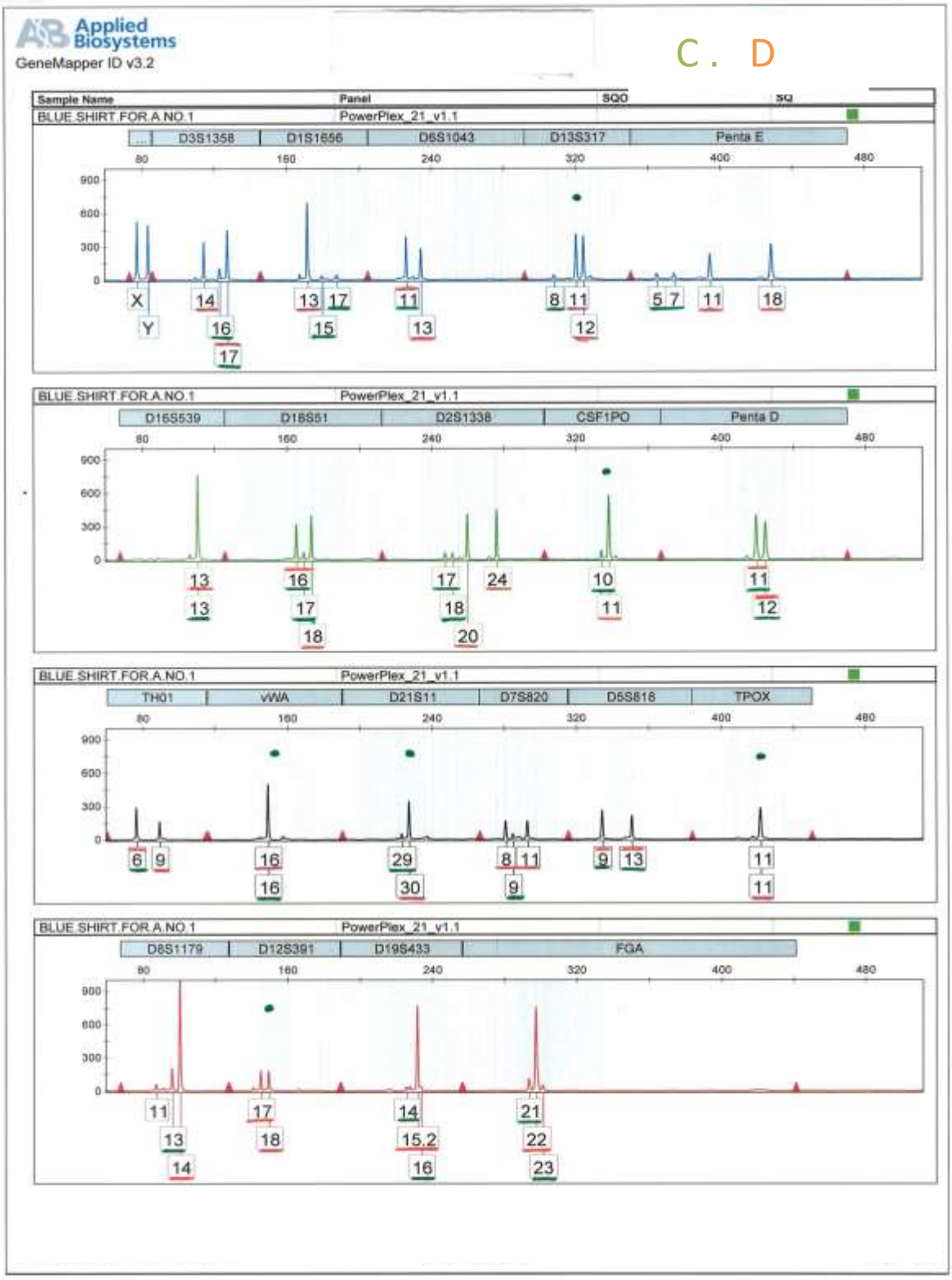

Figure 4: DNA profile for blue shirt A.

Comparison of Allelic Numbers of DNA profiles Between Victim A, Suspects B, C, D and the Clothes for Victim A

The comparison between FTA for victim A, suspects and brown underwear for victim A throughout estimation of allelic numbers were applied in table 5.

Table 5: Show the numbers of alleles in DNA profiles for A,B,C,D and brown underwear A

Sample / Loci	FTA A	FTA B	FTA C	FTA D	Brown underwear A					
AMELO.	X,X	X,Y	X,Y	X,Y	X	Y				
D3S1358	15,17	15,15	16,17	14,17	14	15	16	17		
D1S1656	14,17	16,16	15,17	13,13	13	14	15	17		
D13S317	9,12	8,12	8,13	11,12	8	9	11	12	13	
Penta E	10,13	13,16	5,7	11,18	5	7	10	11	13	18
D16S539	8,8	11,12	13,13	13,13	8	13				
D18S51	13,14	14,18	16,17	16,18	13	14		16	17	18
D2S1338	17,18	20,25	17,18	20,24	17	18	20	24		
CSF1PO	11,12	11,12	10,12	11,11	10	11	12			
Penta D	10,10	9,9	11,12	11,12	10	11	12			
TH01	6,6	6,7	6,6	6,9	6	9				
vWA	16,18	16,19	16,18	16,16	16	18				
D21S11	30,31.2	28,30.2	29,32.2	30,30	29	30	31.2	32.2		
D7S820	10,10	8,10	9,9	8,11	8	9	10	11		
D5S818	11,13	11,11	9,13	9,13	9	13	11			
TPOX	8,11	8,10	8,10	11,11	8	11	10			
D8S1179	11,12	12,15	13,13	14,14	11	12	13	14		
D12S391	18,19	19,19	16,22	17,18	16	17	18	19	22	
D19S433	12,15	13.2,15.2	14,16	15.2,15.2	12	14	15	15.2	16	
FGA	23,25	22,22	21,23	22,22	21	22	23	25		

- Coloured dotes that is noted in DNA profiles are referred to partial (incomplete) profile.

The results show that:

- The DNA profiles were obtained for the samples no. 1,5,6 and 7 that belong to FTA cards for victim A, suspects (B,C and D) respectively .
- For the sample no. 2 (brown underwear A), a mixed DNA profile was obtained that is belong to more than one persons (contain all genetic markers for victim A and suspects C and D).

On the other hand, the comparison between FTA for victim A, suspects B, C, D and blue jeans and shirt for victim A were illustrated in table 6.

Table 6: show the numbers of alleles in DNA profiles for A, B, C ,D, blue jeans and shirt A

Sample / Loci	FTA A	FTA B	FTA C	FTA D	Blue jeans A				Blue shirt A			
AMELO.	X,X	X,Y	X,Y	X,Y	X	Y			X	Y		
D3S1358	15,17	15,15	16,17	14,17	15	16	17		14	16	17	
D1S1656	14,17	16,16	15,17	13,13	14	16	17		13	15	17	
D13S317	9,12	8,12	8,13	11,12	9	12	13		8	11	12	
Penta E	10,13	13,16	5,7	11,18	8	10	13	18	5	7	11	18
D16S539	8,8	11,12	13,13	13,13	8	11			13	13		
D18S51	13,14	14,18	16,17	16,18	13	14	15		16	17	18	
D2S1338	17,18	20,25	17,18	20,24	17	18	20	22	17	18	20	24
CSF1PO	11,12	11,12	10,12	11,11	10	11	12		10	11		
Penta D	10,10	9,9	11,12	11,12	9	10	13		11	12		
TH01	6,6	6,7	6,6	6,9	6	7			6	9		
Vwa	16,18	16,19	16,18	16,16	16	17	18		16	16		
D21S11	30,31.2	28,30.2	29,32.2	30,30	29	30	31.2		29	30		
D7S820	10,10	8,10	9,9	8,11	10	11	13		8	9	11	
D5S818	11,13	11,11	9,13	9,13	11	13			9	13		
TPOX	8,11	8,10	8,10	11,11	9	11			11	11		
D8S1179	11,12	12,15	13,13	14,14	11	12	14		13	14		
D12S391	18,19	19,19	16,22	17,18	18	19			17	18		
D19S433	12,15	13.2,15.2	14,16	15.2,15.2	12	15	15.2		14	15.2	16	
FGA	23,25	22,22	21,23	22,22	22	23	25		21	22	23	

The results show that:

- For the sample no.3 (long blue shirt A), a mixed DNA profile was obtained that is match the DNA profile for suspect D as (a major contributor) and partially match (inconclusive) the DNA profile for the suspect C as (a minor contributor).
- For sample no.4 (blue jeans trousers A), a mixed DNA profile was obtained that is match (inclusion) the DNA profile for victim A and DNA profile that is not match (exclusion) the DNA profiles for suspects B, C and D and belong to unknown person (Bieber *et al.,* 2016) .

The DNA profile for brown underwear (Figure 2, table 5) showed some loci with minimum allelic number equal to 2 (D16S639, TH01, VWA and DYS391) while other loci showed maximum allelic number equal to 6 (Penta E and D18S51), which means that the mixed profile belonged to at least 3 contributors, now we have 2 hypotheses; either this profile belongs to the victim A with 2 suspects (may be known or unknown) or it belongs to 3 suspects (also may be known or unknown) from table (5) we showed that the numbers of loci matched (inclusion) the DNA profile for victim A ,suspects C and D. while no match was found for suspect B ,and was thus excluded from the mixed profile. The DNA profile for blue shirt (Figure 4, table 6) showed minimum allelic number equal to 2 (D6S1043, CSF1P0, Penta D, TH01, D5S818 and D12S391)and maximum allelic number equal to 4 (Penta E and D2S1338), so the profile belongs to 2 contributors ,2 hypotheses can be made; either the mixed profile belongs to victim A and one suspect (may be known or unknown), or the mixed profile belongs to 2 suspect (also may be known or unknown) and the numbers of loci showed matching (inclusion) to DNA profile for suspect D and a partial match (inconclusive) to the DNA profile for suspect C, while suspect B does not appear (exclusion) in the mixed profile (Butler and Gittelson , 2015; Lohmueller and Inman, 2018).

The DNA profile for the blue jeans (Figure 3, table 6) also showed minimum number of loci equal to 2 (D16S539, TH01, D21S11, D5S818, TPOX and D22S1045) and maximum number of loci equal to 4 (D1S1656, D10S1248, Penta E and D2S1338), this means we have 2 contributors; either victim A with one suspect (may be known or unknown) or 2 suspects (also may be known or unknown), the DNA mixed profile in table (6) showed allelic numbers that is matched (inclusion) to the DNA profile for victim A and does not match (exclusion) the DNA profiles for suspects B, C and D and belonged to unknown person (Butler, 2015; Marsden *et al.,*2016).

Table 7: Shows the summary for the interpretation.

Samples / Subject	Brown underwear A	Blue jeans A	Blue shirt A
Victim A	A	A	----------
Suspect B	----------	----------	----------
Suspect C	C	----------	C partial
Suspect D	D	----------	D
Unknown person	----------	UNKNOWN	----------

CONCLUSIONS

Given the results obtained by studying our case, it is safe to conclude that:

DNA using in forensic investigations to produce legally useful evidence when presented with mixed DNA samples from the saliva, vaginal swabs and rectal swabs that comprise DNA from several minor contributors, such as in cases of group rape. Despite the significant advancements achieved, ongoing enhancements in combinatorial methods are required to account for all the challenges, including those presented by stutter, contamination and artifacts of allelic drop out.

CONFLICT OF INTEREST

None

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