Research Journal of Forensic Sciences ISSN 2321–1792
Vol. **11(2),** 15-21, July **(2023)** *Res. J. Forensic Sci.*

Case Study

Calculation of paternity index in a paternity dispute case in Himachal Pradesh, India

Naresh Kumar*, Aishwarya Thakur and Hem Raj
DNA Unit, Regional Forensic Science Laboratory, Mandi, Himachal Pradesh, India
nareshkumarbiotech85@gmail.com

Available online at: www.isca.in, www.isca.me
Received 19th April 2023, revised 17th June 2023, accepted 20th July 2023

Abstract

In forensic science laboratories, DNA testing in disputed paternity cases is a common analysis. The goal of the test is to show the similarities and differences in analyzed genetic markers between the putative father, mother, and child. When the DNA profile of alleged father matches with one of the two alleles examined in the DNA profile of putative child, alleged cannot be excluded as biological father. In the present investigation, the paternity was determined from an aborted foetus. The blood sample of victim and accused collected on FTA cards and foetus were received in the DNA Unit, Regional Forensic Science Laboratory, Mandi to establish the paternity. A full and concordant match was found between the DNA profiles obtained from the accused's blood sample on the FTA card and one of the two alleles in the genotype of the foetus, thus proving paternity. The likelihood of paternity was determined to be >99.99% based on the combined paternity index, which was assessed to be 1.20 x 10^{11}. Therefore, it was impossible to rule out the accused as the biological father of the aborted foetus.

Keywords: Combined paternity index, DNA, FTA card, Paternity.

Introduction

Jeffreys et al.[1] were the first to describe DNA profiling or fingerprinting. However, at that time the application of DNA fingerprinting was in its infancy. With the advancements in short tandem repeats (STR) kits, modern DNA profiling technology has created new opportunities for performing human identity testing in heinous crimes like murder, sexual assault, identifying unidentified dead bodies, etc.[2-5]. In addition, DNA typing technology has the capacity to resolve paternity disputes, and family related issues that could otherwise go unresolved[6-8]. In forensic science laboratories, DNA testing is a common practice in cases of disputed paternity. The typical process involves comparing autosomal STR markers from the putative child, mother, and putative father[9,10].

The Paternity Testing Commission (PTC) of The International Society for Forensic Genetics (ISFG) at Mainz, Germany produced a series of recommendations in 2002 based on ISO 17025 standards for a number of important paternity testing-related topics[11]. In forensic statistics, statistical methods and probability models are applied to scientific data, such as DNA evidence. Juries or judges will then utilise this probability ratio to draw inferences or conclusions in legal matters. Paternity index (PI), one of the forensic statistics parameters, is a likelihood ratio calculated by comparing two probabilities, namely the probability that the tested man is the biological father (X) divided by the probability that a random man chosen from a population (Y) is the biological father[12]. This index increases the statistical significance of forensic DNA report.

In the present case study, the victim knew the accused and both of them desired marriages. She was coerced into engaging in sexual activity by the accused. A few months ago, the accused scheduled a telephone meeting with the victim in a hotel, and engaged in sexual activity without her consent. After a few months, the victim started experiencing abdominal pain, and she told her mother about the incident. Victim's mother admitted her in a hospital and following the victim's medical examination, the medical officer conducted a urine pregnancy test (UPT) to confirm her pregnancy.

When the UPT revealed that she was pregnant, the victim lodged a complaint against the accused in a police station. Later, on the consent of the victim, she delivered a dead foetus in the hospital. After medico-legal examination, the medical officer handed over the police blood sample of victim and accused collected on Flinders Technology Associates (FTA) cards and aborted foetus packed in a jar for paternity determination.

Materials and methods

Materials: The blood samples of victim and accused on FTA cards and an aborted foetus was received in the DNA Unit, Regional Forensic Science Laboratory, Mandi for paternity establishment as a routine laboratory case work through a legal process from one of the police stations of Himachal Pradesh. The victim's and accused's blood sample on FTA cards were marked as "A" and "B" respectively, whereas aborted foetus was labelled as "C".

Methods: The DNA from blood sample of victim and accused on FTA cards was purified as per method of Sahajpal et al.[13] and dried punches were kept at 2-8°C in a freezer (Celfrost, India). Due to ease in collection and handling, the fresh skin tissue of the foetus was chosen for DNA extraction. A small portion of the skin tissue was chopped into pieces with sterilised blade and put into autoclaved micro vial (1.5 ml). Then, DNA was isolated with the Qiagen EZ1 Advanced XL BioRobot utilising a magnetic bead-based technique[14].

As per the manufacturer's instructions, the elution tubes, tip holder having filter-tips, and reagent cartridges were inserted in EZ1® Advanced XL BioRobot (QIAGEN, Hilden, Germany). The "Large-Volume Protocol" was used for DNA isolation. The DNA was stored at 2-8°C in a refrigerator (Celfrost, India) for polymerase chain reaction (PCR) amplification. The extracted DNA from foetus was quantified using Investigator® Quantiplex® Pro RGQ Kit using Rotor-Gene Q real-time thermal cycler (Qiagen, Hilden, Germany). The data were analyzed using PROM-10701-013_Quant Assay Data Handling Tool v4.0 and one nanogram DNA was used for PCR amplification.

PCR Amplification: The extracted and purified DNA was amplified through polymerase chain reaction using GlobalFiler IQC™ kit (ThermoFisher Scientific Inc., U.S.A.). This kit amplifies 21 autosomal short tandem repeats (STR) loci along with amelogenin, and two male-specific markers i.e., Y-Indel and DYS391. The PCR amplification was performed with Veriti™ 96-Well thermal cycler (Applied Biosystems, U.S.A.) as per manufacturer's instructions. The quality of the kit was evaluated by using DNA Control 007 as a positive control and nuclease-free water as a negative control.

Capillary electrophoresis and genotyping: The capillary electrophoresis of amplified PCR products was performed using ABI 3500 Genetic Analyzer (Applied Biosystems, U.S.A.) as per manufacturer's instructions manual. The reaction mixture consisted of Hi-Di formamide (9.6µl), GeneScan 600 LIZ size standard v2.0 (0.4µl), and the allelic ladder (1.0 µl) for each sample.

The run conditions were as follows: Run module: HID36_POP4, Injection conditions: 1.2kV/24 seconds, Run conditions: 13 kV/1550 seconds and Performance Optimized Polymer-4 (POP-4). The genotyping was performed using GeneMapper™ IDX Software v 1.6.

Biostatistical calculations: The following equation was used to determine the paternity index (PI)[15]:

Paternity Index (PI) = X/Y

Where, X = chances of accused is the biological father, Y= any randomly chosen person from the population is the biological father.

The combined paternity index (CPI) was computed by multiplying all PI values. The following formula was used to calculate the probability of paternity:

Probability of paternity = 1/1 + (1/CPI)

The allele frequency of each allele of the relevant marker was selected from the Indian population as per Appendix-A of the Working Procedures Manual for Forensic DNA Testing-2019 of the Central Forensic Science Laboratory, Chandigarh, Directorate of Forensics Services, New Delhi[16].

Results and discussion

The Table-1 displays Punnett square data of the genotypes of the victim (Figure-1), accused (Figure-2), and aborted foetus (Figure-3). As shown in the table, Amelogenin depicted "XX" alleles, whereas no amplification was seen at male specific, Y-Indel and DYS391 markers confirming the gender of aborted foetus (C) as female. On comparison, it was observed that one of the two alleles obtained in the genotype of victim (A) and accused (B) showed match with one of the two alleles in the genotype obtained at all the 21 Autosomal STR loci examined in the aborted foetus, which confirmed that the transfer of paternal and maternal alleles to the foetus following the Mendel's law of inheritance.

This data proved that the accused as the biological father of the aborted foetus. The combined paternity index was assessed to be $1.20x10^{11}$. The probability of paternity was calculated to be 0.9999999999916 (>99.99%) which was significant enough to suggest that the accused cannot be excluded as the biological father of the aborted foetus as compared to randomly chosen, unrelated individual of the Indian population.

Discussion: The DNA technology is important and accurate in solving the cases of paternity disputes as compared to blood grouping. In the current study, paternity of an aborted foetus was established using DNA profiling technology by allelic comparison and calculation of paternity index and combined paternity index. Similar to this study, we observed the combined paternity index (CPI) to be 1,880,0922,263,36 with the probability of paternity of >99.99% in a paternity establishment case[17].

In another study, Liao et al.[18] calculated paternity index from DNA mixture obtained from mother's vaginal tissue contaminated with semen from alleged father establishing a relationship between the alleged father and the child. These studies confirm that paternity index and combined paternity index are important parameters in the cases of paternity disputes, which help in defending the report of forensic DNA analyst in the court of law. Paternity index literature reviews are accessible, but there is insufficient data on case studies to discuss the current findings.

Table-1: The Punnett square table depicting genotypes of victim, accused and aborted foetus.

Markers	Blood sample of victim on FTA card (A)		Blood sample of accused on FTA card (B)		Aborted foetus (C)	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	15	17	15	15	15	15
vWA	14	17	16	17	17	17
D16S539	9	9	9	11	9	9
CSF1PO	12	12	11	11	11	12
TPOX	10	10	8	11	10	11
Y-Indel	-		2		-	
Amelogenin	X	X	X	Y	X	X
D8S1179	13	14	10	15	14	15
D21S11	30	32.2	31.2	32.2	30	31.2
D18S51	14	17	14	14	14	17
DYS391	-		9		-	
D2S441	10	11	14	14	11	14
D19S433	13	14	13	14	13	14
TH01	8	9	6	7	7	8
FGA	21	23	25	25	21	25
D22S1045	11	15	11	15	11	11
D5S818	11	12	10	12	11	12
D13S317	8	12	11	12	8	11
D7S820	10	12	10	11	10	10
SE33	16	26.2	13	16	16	26.2
D10S1248	15	15	14	16	14	15
D1S1656	11	16	14	17	16	17
D12S391	19	20	18	19	19	19
D2S1338	18	23	18	24	23	24

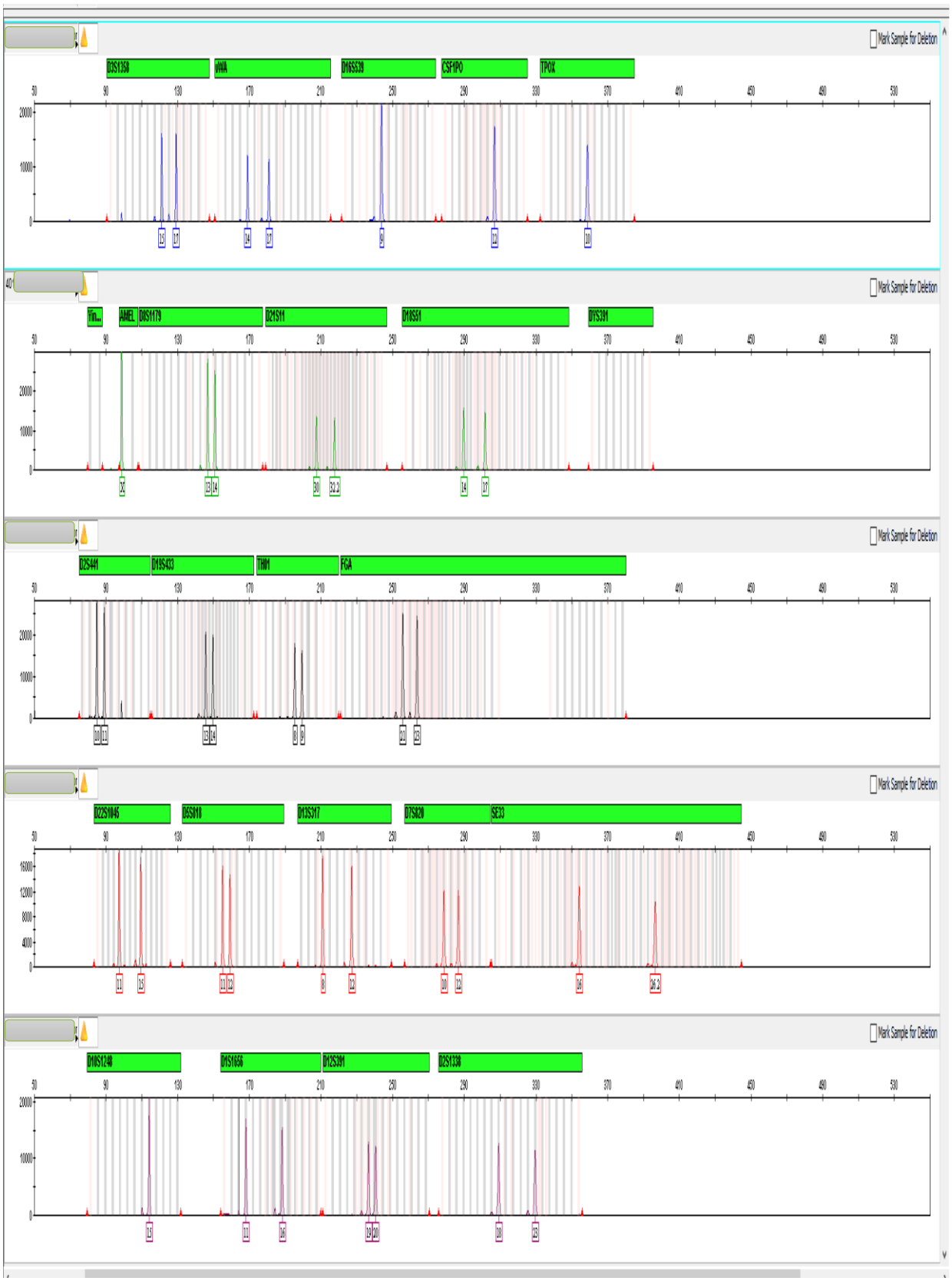

Figure-1: Electropherogram of DNA from blood sample of victim on FTA card (A).

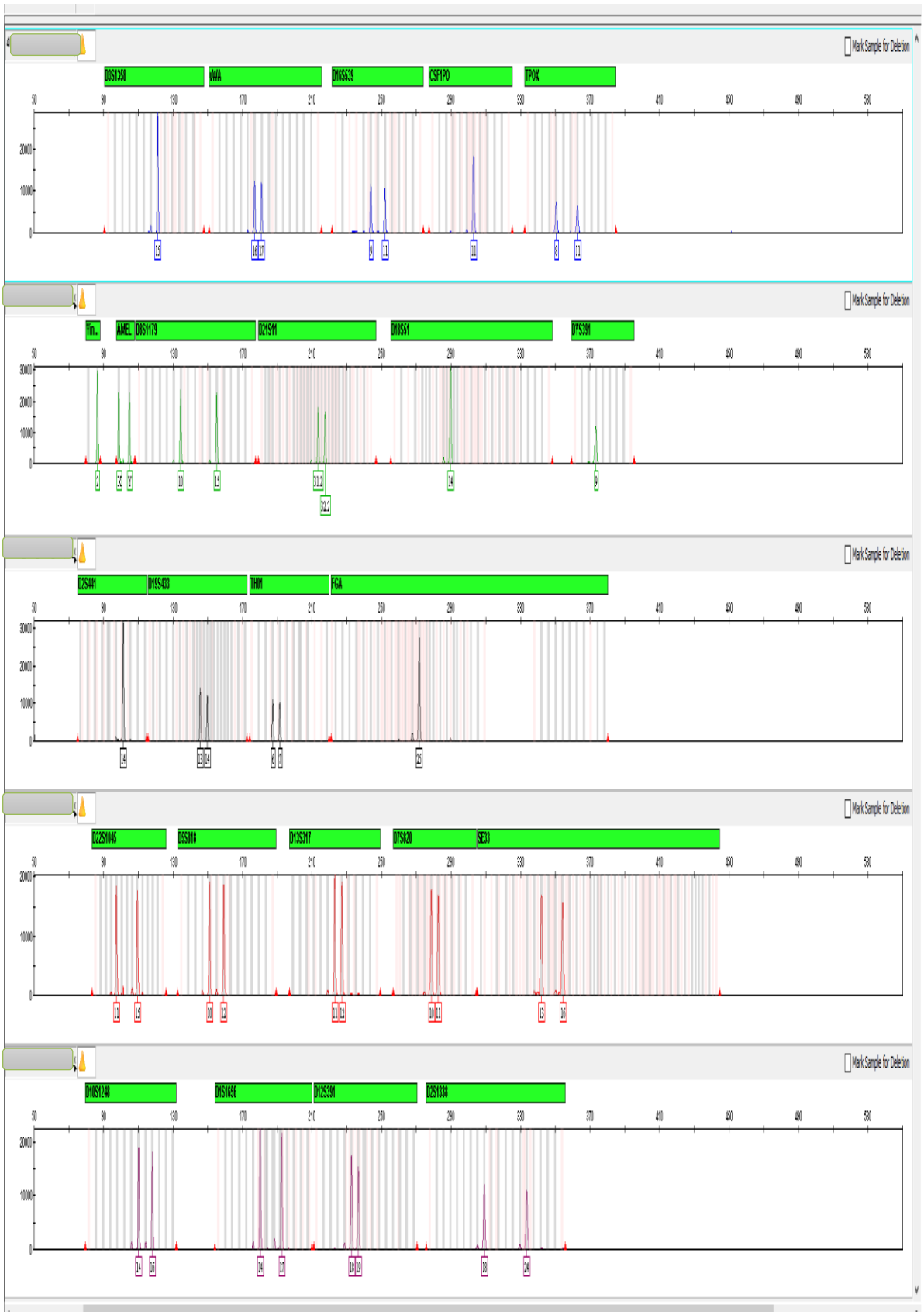

Figure-2: Electropherogram of DNA from blood sample of accused on FTA card (B).

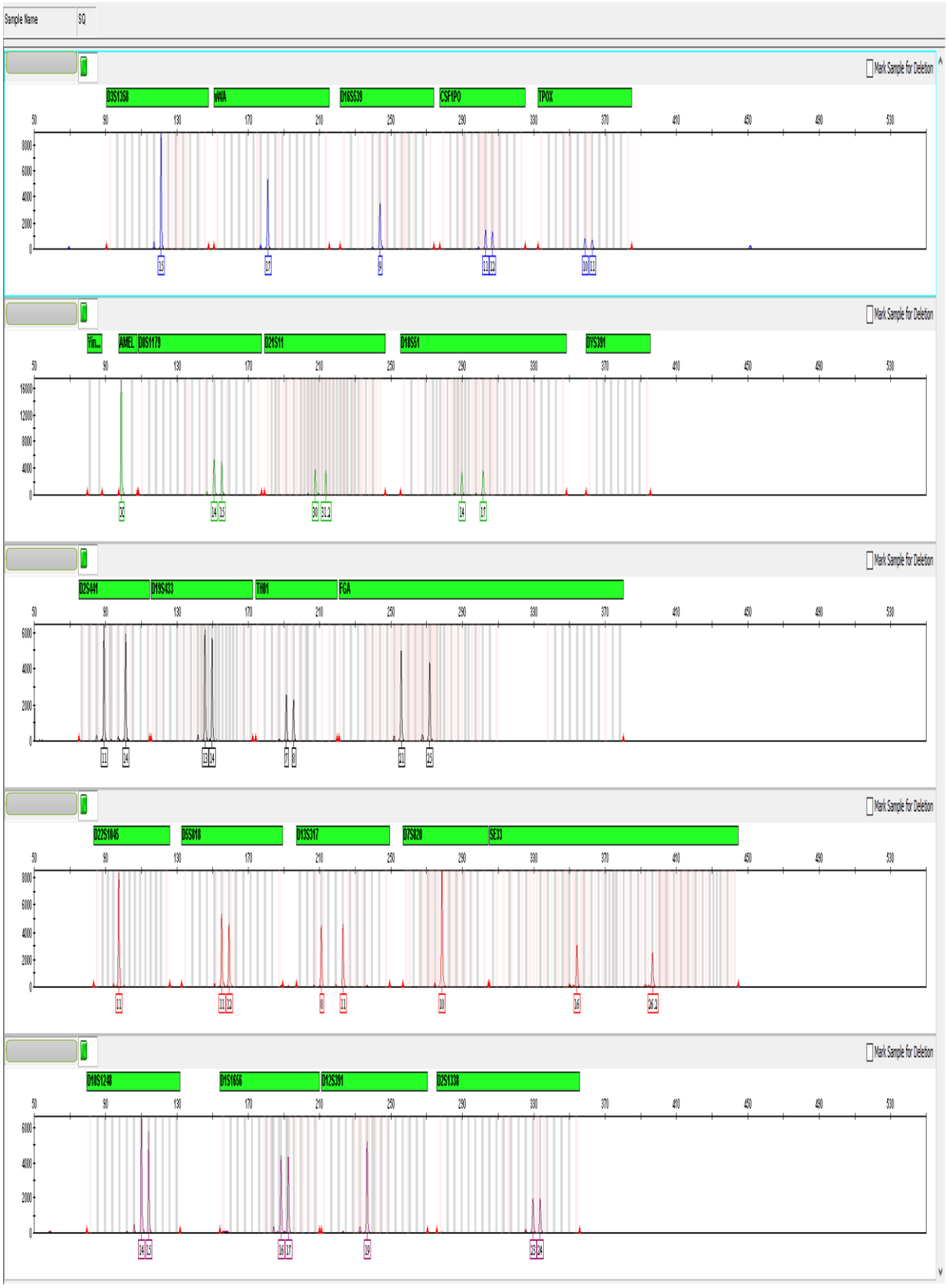

Figure-3: Electropherogram of DNA from aborted foetus (C).

Conclusion

The DNA profiling play an essential role in resolving the cases of murder, sexual assault, paternity/maternity disputes, identification of unidentified dead bodies, etc. in forensic science. For the purpose of determining paternity, the forensic science laboratories accept samples of liquid blood, blood on FTA cards, bones (such as the femur, humerus, sternum, etc.), and foetus with an attached placenta. The proper collection and preservation of samples plays a crucial role in obtaining complete DNA profiles to exonerate the innocent or convict the offenders in the court of law. Moreover, the DNA profiling may benefit from the use of forensic statistics to strengthen the statistical significance of the DNA result. Since, the calculation of the paternity index may be challenging for routine analysis in forensic laboratories, the use of some freely accessible software, such as FamLink, Forensim, EuroForMix, SmartRank, Forensic statistics analysis toolbox (FORSTAT), Commercial off-the-shelf (COTS), Easy DNA, Genolab, Batch Analyzer, Calculate, etc. can be taken into consideration for kinship analysis.

Acknowledgements

The authors are grateful to the Deputy Director, Regional Forensic Science Laboratory, Mandi, Himachal Pradesh to provide necessary facilities to perform this work as a routine case work.

References

1. Jeffreys, A.J., Wilson, V. and Thein, S.L. (1985). Hypervariable 'minisatellite' regions in human DNA. *Nature*, 314, 67-73. https://doi.org/10.1038/314067a0

2. Gill, P., Ivanov, P.L., Kimpton, C., Piercy, R., Benson, N., Tully, G., Evett, I., Hagelberg, E. and Sullivan, K. (1994). Identification of the remains of the Romanov family by DNA analysis. *Nat. Genet*., 6, 130-135.

3. Clayton, T.M., Whitaker, J.P. and Maguire, C.N. (1995). Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. *Forensic Sci. Int.*, 76(1), 7-15.

4. Okamoto, O., Yamamoto, Y., Inagaki, S., Yoshitome, K., Ishikawa, T., Imabayashi, K., Miyaishi, S. and Ishizu, H. (2003). Analysis of short tandem repeat (STR) polymorphisms by the PowerPlex 16 system and capillary electrophoresis: Application to forensic practice (Article). *Acta Medica Okayama*, 57(2), 59-71.

5. Thomson, J.A., Pilotti, V., Stevens, P., Ayres, K.L. and Debenham, P.G. (1999). Validation of short tandem repeat analysis for the investigation of cases of disputed paternity. *Forensic Sci. Int.*, 100(1-2), 1-16.

6. El-Alfy, S.H. and El-Hafez, A.F.A. (2012). Paternity testing and forensic DNA typing by multiplex STR analysis using ABI PRISM 310 Genetic Analyzer. *J. Genet. Eng. Biotech.*, 10(1), 101-112.

7. Torroni, A., Achilli, A., Macaulay, V., Richards, M. and Bandelt, H.J. (2006). Harvesting the fruit of the human mtDNA tree. *Trends Genet.*, 22(6), 339-345.

8. Rodig, H., Roewer, L., Gross, A., Richter, T., De Knijff, P., Kayser, M. and Brabetz, W. (2008). Evaluation of haplotype discrimination capacity of 35 Y-chromosomal short tandem repeat loci. *Forensic Sci. Int.*, 174(2-3), 182-188.

9. Doniec, A., Luczak, W., Wrobel, M., Janula, M., Ossowski, A., Grzmil, P. and Kupiec, T. (2021). Confirmation of paternity despite three genetic incompatibilities at chromosome 2. *Genes (Basel)*, 12(1), 62.

10. Ayres, K.L. (2000). Relatedness testing in subdivided populations. *Forensic Sci. Int.*, 114(2), 107-115.

11. Schneider, P.M. (2007). Scientific standards for studies in forensic genetics. *Forensic Sci. Int.*, 165(2-3), 238-243.

12. Baur, M.P., Elston, R.C., Gürtler, H., Henningsen, K., Hummel, K., Matsumoto, H., Mayr, W. and Moris, J.W. (1986). No fallacies in the formulation of the paternity index. *Am. J. Hum. Genet.*, 39(4), 528-36.

13. Sahajpal, V., Rajput, S., Sharma, T., Sharma, A. and Thakar, M. (2019). Development and evaluation of a novel DNA purification buffer and protocol for blood samples on FTA cards. *Forensic Sci. Int. Rep.*, 1, 100014. https://doi.org/10.1016/j.fsir.2019.100014

14. EZ1&2® DNA Investigator® Kit Handbook. (2022). https://www.qiagen.com/us/products/human-id-and-forensics/investigator-solutions/ez1-2-dna-investigator-kit. Accessed 12 April 2023.

15. Dash, H. R., Shrivastava, P., Das, S., Dash, H. R., Shrivastava, P., & Das, S. (2020). Calculation of Paternity Index in Paternity Dispute and Identification Cases. Principles and Practices of DNA Analysis: A Laboratory Manual for Forensic DNA Typing, 239-251.

16. Working Procedures Manual Forensic DNA Testing (2019). http://dfs.nic.in/pdfs/DNA%20manual%20final%20aug%202019%20(1)-merged%20(1).pdf. Accessed 12 April 2023.

17. Kumar, N. and Sharma, A. (2021). Human identification through DNA analysis of teeth using powder-free method – A case study. *J. Forensic Sci.*, 1, 45-52.

18. Liao, X.H., Lau, T.S., Ngan, K.F.N. and Wan, J. (2002). Deduction of paternity index from DNA mixture. 3 *Forensic Sci. Int.*, 128(3), 105-107.