# DNA Evidence Helps Accused Prove His Innocence

Vaishali B. Mahajan[1*], K. R. Gorle[2], A. A. Pande[3], R. B. Kotpalliwar[4]

[1,3]Assistant Chemical Analyser, [2]Assistant Director, [4]Deputy Director

DNA Fingerprinting Unit, Regional Forensic Science Laboratory, Nagpur [MH], Department of Home Ministry

Government of Maharashtra, Maharashtra, INDIA.

[*]Corresponding Address:

vaishalimahajan.nagpur@gmail.com

***Case Report***

***Abstract:*** Three innocent youths falsely implicated in the crime of rape u/s 376 of Indian Penal Code r/w section 3 (1) (12) of Prevention of Scheduled Caste Scheduled Tribe Atrocity Act and detained in police / magisterial custody could be saved from being punished for the crime not at all committed by them only with the help of non refutable evidence in the form of DNA profile.

***Keywords:*** DNA Fingerprinting, Kastle-Meyer (Phenolphtahalein) reagent.

## Introduction

In the investigation of crime the DNA fingerprinting technology is a powerful tool in the hands of forensic biologists as it not only helps to prove guilt of the accused but his innocence too. Biological evidences are the main sources of DNA. The DNA is isolated from different biological sources, such as blood, semen, vaginal swab, bone, tissue, hair etc. in criminal cases like sexual offences, murder and other offences affecting human body. In the instant case, a girl aged 18 from a small village in Bhandara district of Maharashtra, India lodged a complaint with police station alleging that while she was returning home from shop, three persons from the same village whose names she mentioned in her complaint, picked her up, shut her mouth and forcibly carried her to a nearby lake with muddy earth and raped her one after the other. Contrary to the allegation made by the complainant, all the accused firmly refuted the charges of rape leveled against them and further even refused to be released on bail. During the course of investigation, investigating officer submitted the clothes of the complainant and of all the three accused worn by them at the time of alleged rape. Besides clothing, the articles i.e. blood samples, pubic hair of all parties and vaginal swab of complainant drawn by the medical officer at the time of their medical examination were also submitted. During the course of analysis in Regional Forensic Science Laboratory, Nagpur, semen stains were detected on the clothes of the complainant. The DNA profiles of all these semen stains were found to be identical implying a single perpetrator to be the source of the semen but surprisingly failed to match with the DNA profiles of any of the three accused. Meanwhile, the police received information and on further investigation it was revealed to the police that the complainant had affairs with another boy from the same village since long and at the time of alleged rape they had been together for quite a long time. The said boy was absconding since then. The police tried their level best to apprehend him and were successful in their attempt after sometime. His blood sample was sent for DNA profiling. On analysis of blood, it was revealed that the DNA profile of his blood sample exactly matched with the DNA profile of the semen stains detected on the clothes of the complainant. The DNA profiling technique in the instant case would help absolve all the innocent accused of the charges leveled against them, otherwise would have been very difficult for them to prove their innocence in the offence like rape and that too with certain sections of Atrocity Act, where onus lies on the accused to prove their innocence.

## Procedures for detection of biological stains and extraction, quantification, amplification and electrophoresis of DNA

## Materials and Method

Kastle-Meyer (Phenolphtahalein) reagent
Florence reagents
Acidulated water containing 1 one drop of con HCl in 44 ml of distilled water
Citrate buffer (pH 4.9)
Disodium phenyl phosphate
4 amino antipyrene solution
Pottaciun Ferrycynide solution
4% EDTA
FTA CARD
FTA Purification reagent
TE Buffer (pH-8)
Forensic Buffer ( 1M Tris HCl, 0.5M EDTA, 5M NaCl)
10% SDS
Proteinase K
40mMdTT
Phenol:Choloroform:Isoamyl alcohol (25:24:1)
Isopropanol

70% alcohol
AmpFlSTR PCR Reaction Mix
AmpliTaq Gold DNA polymerase
AmpFlSTR Primer set
Formamide
Size standard
Allelic ladder

## Process

Detection of biological stains and collection of blood samples
⇓
Preservation of samples
⇓
Extraction of DNA
⇓
Quantification of DNA
⇓
Amplification of DNA using PCR
⇓
Electrophoresis
⇓
Analysis of STR Products
⇓
Conclusion

Clothes of victim and all the three accused sent for analysis in the laboratory were checked for the presence of biological specimen like blood and semen. For detection of blood on the clothes of victim and accused, Kastle-Meyer (Phenolphthalein) test was employed. The test was negative for all the exhibits except on the knicker of the victim. Kastle-Meyer test, though not being a confirmatory test for blood, excludes the possibility of presence of blood if reaction is found to be negative. Blood was also not detected on the vaginal swab of the victim. Few small washed blood stains were detected on the knicker of victim which were confirmed to be of human origin by crossed over electrophoresis method. The aforesaid articles were also checked for the presence of semen. For detection of semen two tests namely Acid Phosphatase test and Florence test were employed. Acid phosphatase test detects the presence of acid phosphatase enzyme, whereas Florence test detects the presence of choline, and these two substances i.e. acid phosphatase and choline together are present only in semen. Semen stains were detected on the salwar and knicker of the victim and knickers of the two of the accused. Semen was also detected on the vaginal swab of victim.

All the above semen stains were taken for DNA typing. Blood samples of victim and all accused were called for. Different procedures were employed for the extraction of DNA from the above biological specimen as under

1. For semen stains on clothes of victim and accused and vaginal swab of victim **:** Differential extraction method, which is a modified version of organic extraction method.( Sperm nuclei are lysed using dithiothretol).
2. For blood samples of victim and accused**:** FTA Paper method was used.

## Quantification of DNA

The quantity of DNA was checked by gel electrophoresis by comparing the DNA of the sample in question with DNA of known molecular weight. DNA extracted from semen stains on the garments of victim, accused, vaginal swab of victim and blood samples of victim and accused was amplified by using Polymerase Chain Reaction technique. By this process short segments of DNA sequence i.e. short tandem repeats (STR) are selectively replicated a million fold or more in about 28 cycles. During the process initially DNA samples were incubated at 95°C for 11 minutes. Thereafter DNA was amplified in 28 cycles selecting 94°C, 54°C and 72°C as a temperature of denaturing, annealing and extension respectively. Amplified samples of DNA were then kept at 60°C for an hour and then at 4°C till separation of STRs. Polymerase chain reaction (PCR) produces millions of copies of DNA fragments of different sizes. Separation of the different fragments of DNA molecules on the basis of their size was achieved by capillary electrophoresis. The instrument, ABI Prism 3130 Genetic Analyser (Applied Biosystems), was used for carrying capillary electrophoresis. Only single stranded DNA fragments are resolved by this instrument. Therefore, the amplified DNA samples were denatured by heating the samples at 95°C for 3 minutes and then by snap cooling at 0°C (ice). To keep the DNA molecule single stranded throughout the process of electrophoresis, before injecting the samples, they were diluted with formamide. The voltage employed for separation of DNA fragment was 15000V. The separated fragments of DNA molecules were detected by fluorescence detector. The DNA profiles thus obtained were compared with each other and their results were as under.

## Results and Discussion

### DNA Profiling Evidence

DNA extracted from the semen stains detected on clothes and vaginal swab of complainant, knickers of accused 2 and 3 and blood samples of all the accused along with blood of complainant's friend was typed at 15 STR LOCI and gender specific Amelogenin locus using PCR Amplification technique.

Results of analysis are summarized in the table 1 below.

**Table 1:** Results of DNA profiling evidence

| STR LOCUS | Semen stains detected on | | | | | | | | Standard blood samples of | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salwar 0f Complainant | Knicker of Complainant | | | | Knicker of accused no. 2 | Knicker of accused no. 3 | Vaginal Swab of Complainant | accused no. 1 | accused no.3 | accused no.2 | complainant's friend |
| | | A | B | C | D | | | | | | | |
| D8S1179 | 11,15 | 11,15 | 11,15 | 11,15 | 11,15 | 10,12 | 11,12 | 11,15 | 12,15 | 11,12 | 10,12 | 11,15 |
| D21S11 | 29,32 | 29,32 | 29,32 | 29,32 | 29,32 | 29,31.2 | 31.2,32.2 | 29,32 | 31.2,32.2 | 31.2,32.2 | 29,31.2 | 29,32 |
| D7S820 | 9,11 | 9,11 | 9,11 | 9,11 | 9,11 | 8,11 | 8,8 | 9,11 | 11,11 | 8,8 | 8,11 | 9,11 |
| CSF1PO | 11,13 | 11,13 | 11,13 | 11,13 | 11,13 | 10,12 | 10,11 | 11,13 | 10,12 | 10,11 | 10,12 | 11,13 |
| D3S1358 | 17,17 | 17,17 | 17,17 | 17,17 | 17,17 | 15,17 | 13,16 | 17,17 | 17,18 | 13,16 | 15,17 | 17,17 |
| THO1 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 6,9 | 6,9.3 | 7,9 | 6,6 | 6,9.3 | 6,9 | 7,9 |
| D13S317 | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 | 8,12 | 11,12 | 11,13 | 8,12 | 11,12 | 11,12 |
| D16S539 | 11,11 | 11,11 | 11,11 | 11,11 | 11,11 | 11,13 | 12,13 | 11,11 | 10,12 | 12,13 | 11,13 | 11,11 |
| D2S1338 | 18,19 | 18,19 | 18,19 | 18,19 | 18,19 | 20,20 | 19,20 | 18,19 | 23,24 | 19,20 | 20,20 | 18,19 |
| D19S433 | 16,18 | 16,18 | 16,18 | 16,18 | 16,18 | 14,14.2 | 14,15.2 | 16,18 | 13,14 | 14,15.2 | 14,14.2 | 16,18 |
| Vwa | 17,18 | 17,18 | 17,18 | 17,18 | 17,18 | 14,14 | 17,19 | 17,18 | 15,16 | 17,19 | 14,14 | 17,18 |
| TPOX | 10,11 | 10,11 | 10,11 | 10,11 | 10,11 | 9,11 | 8,9 | 10,11 | 8,8 | 8,9 | 9,11 | 10,11 |
| D18S51 | 12,16 | 12,16 | 12,16 | 12,16 | 12,16 | 14,16 | 13,14 | 12,16 | 13,19 | 13,14 | 14,16 | 12,16 |
| AMELO GENIN | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y | X,Y |
| D5S818 | 10,12 | 10,12 | 10,12 | 10,12 | 10,12 | 10,12 | 10,11 | 10,12 | 10,13 | 10,11 | 10,12 | 10,12 |
| FGA | 23,23 | 23,23 | 23,23 | 23,23 | 23,23 | 22,23 | 24,24 | 23,23 | 20,24 | 24,24 | 22,23 | 23,23 |

All the four semen stains detected on the knicker of the complainant and a lone semen stain detected on her salwar and semen detected on her vaginal swab gave identical DNA profile, which indicates the involvement of a single person as source of said biological material i.e. semen. DNA profile of semen stains detected on the knickers of accused no. 2 and 3 exactly matched with respective DNA profile of their blood. However, the DNA profile of the semen stains detected on clothes and vaginal swab of complainant failed to match with DNA profiles of all the three accused indicating their involvement in the alleged crime as improbable. It seems that the complaint lodged by the complainant is based on a concocted story to implicate the accused for the purpose best known to her. Matching of DNA profile of the complainant's friend with DNA profile of all the semen stains detected on complainant's clothes and her vaginal swab establishes his accountability for the semen stains on her clothes and vaginal swab and thereby exonerate all the accused of the charges leveled against them.

## Conclusion

DNA STR profiling, a boon to the upcoming forensic era is also a powerful weapon and needs to be used very scrupulously. DNA fingerprinting technique is a double edged weapon. Which on one hand provides irrefutable evidence to punish the perpetrator and at the same time it also helps to prove innocence of a person who is falsely implicated. In the instant case all the corroborative evidences were against the accused. Semen was detected on the clothes and vaginal swab of the complainant. By the conventional methods of analysis, it was rather difficult to exclude the accused as the source of semen found on the clothes and vaginal swab of the complainant. It is only for the DNA analysis; miscarriage of justice could be prevented by proving the innocence of all the three accused. It is for the DNA profiling technology, the people with malicious intention would not dare to file the false complaint on the basis of the concocted stories as in the instant case.

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