



IJEAST

INTERNATIONAL JOURNAL
OF ENGINEERING APPLIED SCIENCE
AND TECHNOLOGY



VOLUME : 7 ISSUE : 04 Print / Issue Publication Date: 30-Sep-2022



ISSN : 2455-2143



DOI : 10.33564/IJEAST.2022.v07i04.014

Indexed In



WWW.IJEAST.COM

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MULTIDISCIPLINARY FORENSIC ANALYSIS ACTS AS AN EYEWITNESS TO LINK THE CRIME- CULPRIT- CRIME SCENE- DECEASED. - AN INTERESTING CASE STUDY.

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Abstract: Different types of evidence found on the crime scene can be classified into biological, chemical, physical, and digital. Biological evidence is a person's body fluids, hair, tissue, etc, chemical evidence is any material used in the crime such as poison, drugs, inflammable substances, chili powder, etc, objects such as weapons, soil, fibers, broken glass piece, etc constitute physical evidence and digital evidence includes CCTV footage, online purchase history of weapons, mobile phones, pen drive, etc. These evidences are analyzed by different disciplines of forensic science like Biology, DNA, Chemistry, Toxicology, Ballistics, and Cyber. In a gruesome murder that took place in broad daylight, the victim's car was surrounded by a few people at a traffic signal. Car's window glasses were broken and he was brutally murdered using sharp-edged weapons. Forensic Science Laboratory helped the investigating agency to collect evidences at the crime scene. Almost every

discipline of forensic science was involved in the analysis of the evidences found (biological, chemical, physical and digital). Detection, assessment and interpretation of the evidences aided to fix the crime scene, the presence of the accused on the crime scene and his involvement in the crime. This multidisciplinary analysis freed the investigating agency from dependency on eyewitnesses. Thus, scientific evidence from various disciplines of forensic science contributed their part and completed the whole picture of murder.

Keywords: Crime scene, scientific evidence, bloodstains, Face mask DNA, physical matching, glass pieces, video analysis, CCTV footage, magazine and pistol matching.

Physical evidence cannot be intimidated. It does not forget. It sits there and waits to be detected, preserved, evaluated and explained. – Herbert Leon Macdonell.



I. INTRODUCTION:

Forensic investigation of a crime is based on a principle came to be known as Locard's Exchange Principle. It implies that whenever a crime is committed, the perpetrator leaves behind something of his own and carries with him something from the scene. The investigators should apply their knowledge, training and experience to recognize and gather evidence including traces that may yield clues or the potential to identify culprits. With the help of scientific evidence, theories regarding happenings at the crime scene can be made or refuted by the logical application of the facts. Management of crime scene, identification of evidence, its location, collection and preservation and proper documentation are the critical issues in investigating any crime. During the investigation of a crime, the aim should not be only to procure necessary information to carry out legal proceedings but also to try to obtain the facts that will result in solving the crime that is to focus on acquiring the details that can confirm the crime and the culprit [1]. Processing the crime scene as early as possible makes it possible to evaluate the most critical aspects of the crime [2].

In the instant case, the victim while driving a car was stopped at the road signal. A crowd of about seven people encircled his car with their vehicles. Some of them broke the window of the car and threw chili powder at the victim and stabbed him with sharp-edged weapons. This attack resulted in the spot death of the victim. One of the accused carried a pistol with him.

Articles for forensic analysis were collected from different sources such as the crime scene, the car of the victim, clothes of the victim, clothes and weapons from the culprits, facemasks from the vehicle of the culprit, and CCTV footage from the crime scene and nearby area. Forensic laboratory officers helped the investigating agency to collect the trace evidences. Articles collected from different sources were as follows:

Crime scene: Bloodstained soil, Magazine of the country made pistol, Knife cover, broken glass pieces.

Victim's car: Red-colored powder, broken glass pieces.

Victim: Blood, Nail Clippings, Scalp hair and Clothes of the victim.

Culprit 1: Jeans pant, left foot shoe with sock and right foot shoe with a sock.

Culprit 2: Two knives without cover, two knives with cover, and an axe.

Culprit 3: Jeans pant, Country made pistol without a magazine, right foot shoe and left foot shoe.

Culprit 4: Four face masks from the culprit's vehicle.

Hair, fibers and small glass pieces found on the weapon, and blood samples of all the culprits collected by the medical officer.

CCTV footage of the crime location and nearby location along with reference photographs of the culprits.

All these evidences required examination from different disciplines of forensic science. The articles were referred to respective divisions for the analysis purpose.

Division wise analytical findings are summarized below:

Biology Division:

Crime scene articles, articles collected from the victim's car, victim, and culprits 1, 2, 3, 4 and articles sent by the medical officer were examined for the presence of blood. It is important to determine whether the red-colored stains found on the crime scene or on clothes are blood or not. Generally, everyone recognizes blood due to its color, if fresh it is red and if aged or older, it appears reddish-brown. But not all the stains which appear red-colored are of blood. Articles such as paint, rusty water, and food coloring can appear similar to blood. Therefore, serological presumptive tests are used to know if a particular red drop on the crime scene is indeed human blood. Kastle-Meyer phenolphthalein test confirms that the particular stain is blood and precipitin tests verify that it is human blood [3, 4].

Kastle-Meyer (KM) test, is a fast, affordable and efficient test and therefore extensively used for the detection of blood. The principle behind this test is the peroxidase-like activity of the hemoglobin in blood catalyzes the oxidation of the colorless reduced phenolphthalein into bright pink phenolphthalein. As for the sensitivity of the test, blood up to the dilution of 1:10,000 can be efficiently detected [5].

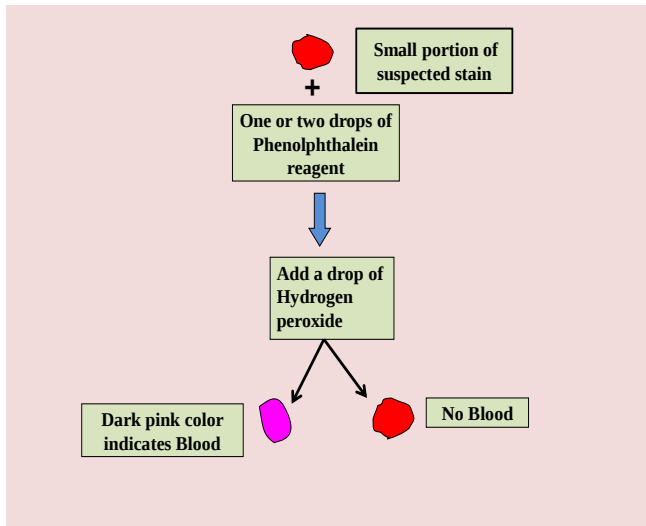


Figure 1: Schematic representation of Kastle Meyer Phenolphthalein test.

For identification of species of blood, i.e. whether it is human or not, a precipitation test is used. Crossed-over Immuno electrophoresis is based upon the interaction of antigen with antibody leading to the formation of antigen-antibody complexes. The antigen-antibody complexes can form cross-linked complexes at the optimal ratio of antigen-to-antibody concentration. This cross-linked complex being insoluble, precipitates and can be observed by the naked eye. This method is a combination of immunodiffusion and electrophoresis. With this technique, a sharp precipitate band is observed for a positive reaction [6].

Antibodies are loaded in wells on the anode side and antigens are loaded in wells on the cathode side. During gel electrophoresis, the antigens that are negatively charged migrate towards the anode. The migration of antibodies is in the opposite direction due to electroendosmosis. A precipitate line or band is formed between opposite wells if there is an antigen-antibody reaction.

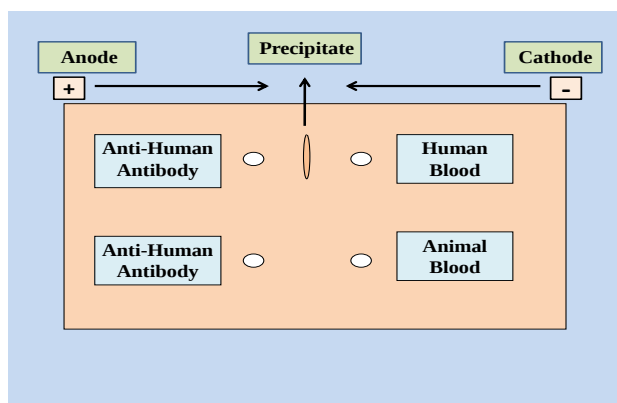


Figure 2: Results of Crossed-Over Electrophoresis

(I) A precipitate band is formed between a blood sample and an antihuman antibody.

(II) Anti-human antibody does not react with animal blood and hence there is no precipitation observed.

Blood-stained articles from the crime scene, victim's car, clothes, bloodstains on weapons, culprit's clothes, the blood of the victim and all the culprits were referred for DNA analysis to DNA division. Articles such as glass pieces, chili powder and knife cover from the crime scene, shoes and weapons (knives) seized from the culprits were referred to General Analytical & Instrumentation division for comparison purpose.

DNA Division:

DNA profiling, one of the eminent discoveries in the 20th century, has transformed criminal investigations. Identification of individuals based on their unique genetic makeup is possible because of state of the art procedure called DNA profiling. The human body is made up of cells and nearly every cell in the human body has the same DNA; therefore DNA in the blood is identical to that in hair, semen, saliva, tissue, and bone [7]. It was found that certain regions of DNA contained repeated DNA sequences. DNA regions with short repeat units (usually 2-6 base pairs in length) are called Short Tandem Repeats (STR). STRs are extensively used in forensic casework across the world during the past few decades [8,9]. Autosomal STRs are highly discriminating, abundantly present in the human genome, have a low mutation rate and smaller amplicon size. These loci have become very useful markers in human identification, parentage testing & population genetic studies [10]. DNA profiling can be divided into four stages:

(I) Extraction of DNA: Biological evidence like blood, saliva, hair, etc. has to be processed to extract DNA. Nowadays robotic extraction methods are used that yield pure DNA. The machines are hassle-free and as the number of sample handling steps is reduced, the risk of contamination reduces greatly. Operational steps include lysis of the samples i.e cells, binding the nucleic acids to the beads, washing and finally eluting the nucleic acids. DNA was extracted from the biological samples using EZ1 Advanced Machine and EZ1 Investigator kits as per the manufacturer's protocol [11].



Figure 3: Robotic extraction of DNA

(II) Quantification of DNA: The extracted DNA has to be quantified prior to processing further. Quantification of DNA solves two main purposes: to determine the proper amount of DNA required for downstream analysis and to preserve the maximum amount of evidence for re-examination.

(III) Amplification of DNA: The DNA quantity that we get after extraction is very less and cannot be detected as such.

For the interpretation purpose, the quantity of DNA has to be amplified and this is achieved through the Polymerase Chain Reaction technique (PCR). PCR allows the production of thousands of millions of copies of DNA from a very small amount of initial DNA. [12]. DNA amplification was performed using Amp FISTR® Identifier kit following the manufacturer's user manual.

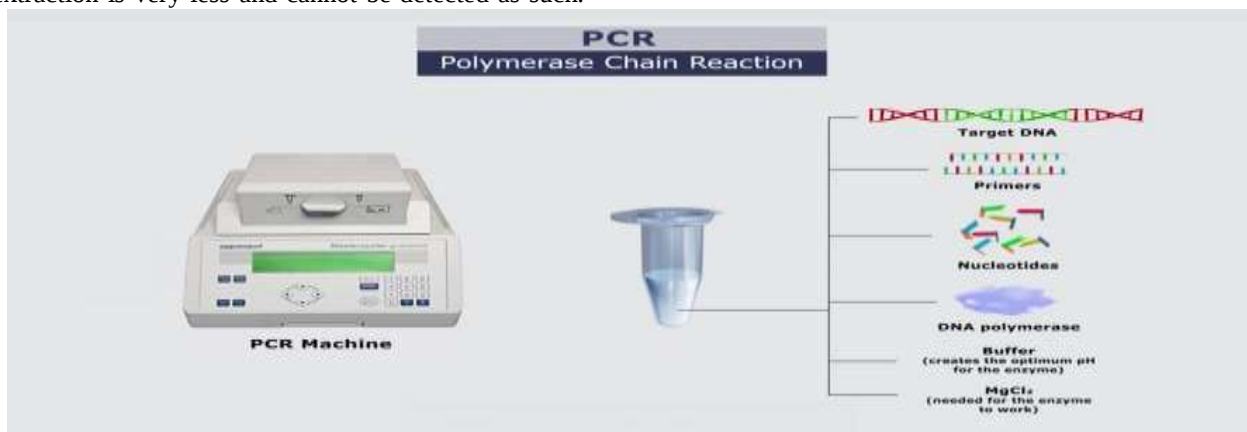


Figure 4: Components of Polymerase Chain Reaction

Steps in thermal cycle of Polymerase Chain Reaction:

	Hold 1	28-30 cycles of 3 temperatures				Hold 2
Temperature °C	95.0	94.0	59.0	72.0	60.0	4.0
Time (mins.)	11.00	1.00	1.00	1.00	60.00	∞
	Initial Denaturation	Denaturation	Annealing	Elongation	Extension	

Amplified products were separated and detected using 3130 Genetic Analyzer [13]. DNA sequencing is achieved through Genetic analyzers which are automated systems. In capillary electrophoresis, DNA fragments attached with dye-labeled primers migrate through a polymer and are hit by a laser at a particular point. The fluorescence that is emitted is measured. The movement of fragments is achieved through Electrokinetic injection which is performed by applying a voltage (kilovolts) at both ends of the capillary. The voltage makes the fragments move through the polymer following the electroosmotic flow. Sixteen STR Loci are simultaneously amplified and analyzed [14, 15].

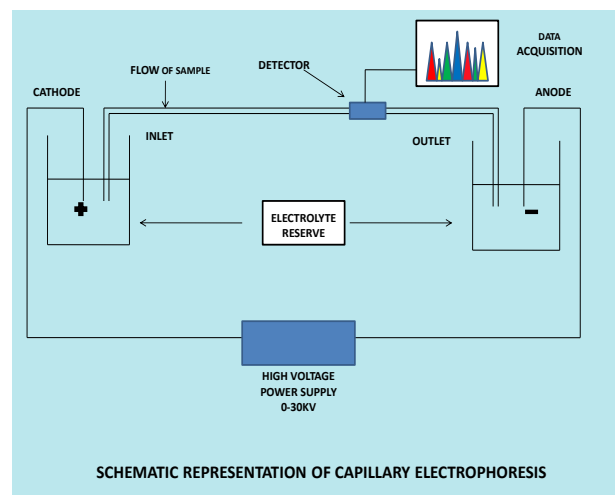


Figure 5: Outline of Capillary Electrophoresis

The DNA extracted from blood stained articles from crime scene, victim's car, clothes, blood stains on weapons, culprit's clothes, blood of victim and all the culprits and face masks recovered from culprit's vehicle was typed at 15



STR loci and gender specific Amelogenin locus using PCR amplification technique.

The results of DNA typing are summarized in the tables below:

STR Loci	GENOTYPE										
	Face Mask 1	Face Mask 2	Face Mask 3	Face Mask 4	Face Mask 4 Blood stain	Crime scene Cotton Swab1 Blood stain	Crime scene Cotton Swab 2 Blood stain	Crime scene Cotton Swab 3 Blood stain	Victim Full Kurt a Blood stain	Victim Full Pant Blood stain	Culprit 1 Left foot shoe with socks Blood stain
D8S1179	10, 12	10, 13	10, 13	12, 14	10, 13	11, 16	11, 16	11, 16	11, 16	11, 16	11, 16
D21S11	29, 31	29.2, 31.2	30, 30	26, 33.2	29.2, 31.2	31.2, 32.2	31.2, 32.2	31.2, 32.2	31.2, 32.2	31.2, 32.2	31.2, 32.2
D7S820	10, 11	8, 10	8, 10	11, 11	8, 10	11, 13	11, 13	11, 13	11, 13	11, 13	11, 13
CSF1PO	10, 13	11, 12	11, 12	12, 13	11, 12	12, 12	12, 12	12, 12	12, 12	12, 12	12, 12
D3S1358	15, 16	15, 18	14, 15	15, 15	15, 18	15, 16	15, 16	15, 16	15, 16	15, 16	15, 16
THO1	9, 9	6, 9	6, 8	7, 9	6, 9	9, 9	9, 9	9, 9	9, 9	9, 9	9, 9
D13S317	11, 11	8, 12	12, 12	11, 12	8, 12	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12
D16S539	8, 12	12, 13	9, 13	9, 13	12, 13	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12
D2S1338	18, 25	22, 25	18, 18	17, 23	22, 25	20, 25	20, 25	20, 25	20, 25	20, 25	20, 25
D19S433	14, 15	13, 15.2	12, 13	13, 14.2	13, 15.2	12, 16.2	12, 16.2	12, 16.2	12, 16.2	12, 16.2	12, 16.2
vWA	14, 14	16, 20	14, 16	15, 18	16, 20	16, 18	16, 18	16, 18	16, 18	16, 18	16, 18
TPOX	8, 9	11, 11	8, 11	9, 11	11, 11	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8
D18S51	16, 16	14, 23	12, 15	16, 16	14, 23	13, 14	13, 14	13, 14	13, 14	13, 14	13, 14
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	10, 13	11, 11	12, 12	11, 12	11, 11	11, 11	11, 11	11, 11	11, 11	11, 11	11, 11
FGA	20, 22	21, 21	22, 23	20, 25	21, 21	23, 25	23, 25	23, 25	23, 25	23, 25	23, 25

Table I: Genotypes of the crime scene articles and blood stained articles at 15 different STR loci and gender specific Amelogenin locus.



STR Loci	GENOTYPE							
	Samples collected by Medical Officer							
	Victim			Blood Culprit 1	Blood Culprit 2	Blood Culprit 3	Blood Culprit 4	Blood Culprit 5
	Blood soaked gauze piece	Scalp hair	Nail clipping					
D8S1179	11, 16	11, 16	11, 16	12, 16	10, 13	10, 12	10, 13	12, 14
D21S11	31.2, 32.2	31.2, 32.2	31.2, 32.2	29, 30	30, 30	29, 31	29.2, 31.2	26, 33.2
D7S820	11, 13	11, 13	11, 13	8, 10	8, 10	10, 11	8, 10	11, 11
CSF1PO	12, 12	12, 12	12, 12	11, 13	11, 12	10, 13	11, 12	12, 13
D3S1358	15, 16	15, 16	15, 16	15, 17	14, 15	15, 16	15, 18	15, 15
THO1	9, 9	9, 9	9, 9	7, 8	6, 8	9, 9	6, 9	7, 9
D13S317	11, 12	11, 12	11, 12	11, 12	12, 12	11, 11	8, 12	11, 12
D16S539	11, 12	11, 12	11, 12	9, 11	9, 13	8, 12	12, 13	9, 13
D2S1338	20, 25	20, 25	20, 25	18, 19	18, 18	18, 25	22, 25	17, 23
D19S433	12, 16.2	12, 16.2	12, 16.2	12, 13	12, 13	14, 15	13, 15.2	13, 14.2
vWA	16, 18	16, 18	16, 18	14, 18	14, 16	14, 14	16, 20	15, 18
TPOX	8, 8	8, 8	8, 8	9, 9	8, 11	8, 9	11, 11	9, 11
D18S51	13, 14	13, 14	13, 14	13, 14	12, 15	16, 16	14, 23	16, 16
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	11, 11	11, 11	11, 11	13, 13	12, 12	10, 13	11, 11	11, 12
FGA	23, 25	23, 25	23, 25	22, 23	22, 23	20, 22	21, 21	20, 25

Table I: Genotypes of the samples of victim and blood samples of culprits collected by medical officer at 15 different STR loci and gender specific Amelogenin locus.

Observation and comparison of DNA profiles revealed that:

- DNA profiles obtained from blood detected on crime scene articles, clothes of victim and left foot shoe with sock of culprit 1 were identical and from one and the same source of male origin and **matched** with DNA profile obtained from blood sample of victim sent by medical officer.
- Male DNA profile obtained from Face mask 1 **matched** with DNA profile obtained from blood sample of Culprit 3.
- Male DNA profile obtained from Face mask 2 **matched** with DNA profile obtained from blood sample of Culprit 4.
- Male DNA profile obtained from Face mask 3 **matched** with DNA profile obtained from blood sample of Culprit 2.

- Male DNA profile obtained from Face mask 4 **matched** with DNA profile obtained from blood sample of Culprit 5.

- Male DNA profile obtained from blood on Face mask 4 **matched** with DNA profile obtained from blood sample of Culprit 4.

General Analytical & Instrumentation Division:

The physical evidence collected from the crime scene like glass pieces and shoes of the culprit were sent for detection and comparison of glass pieces and detection of chili powder. A knife cover found near the victim's car and knives seized from the culprit were sent for physical alignment comparison.

Forensic analysis of Glass:

Glass can be major evidence when it is broken while committing a crime. Small pieces or fragments of broken glass are transferred to and retained by objects or persons nearby the crime. The importance of such evidence is greatly amplified if the fragments are found to be identical to the broken glass [16, 17].

Glass comparison includes the examination of parameters like color/hue, thickness/height and density of the glass. Micro-XRF technique is one of the methods used to know the elemental composition of the glass fragments [18]. Micro X-ray fluorescence (μ XRF) is an elemental analysis technique that allows for the examination of very small sample areas. In micro X-ray Fluorescence, direct X-ray excitation is used to induce characteristic X-ray fluorescence emission from the sample. An X-ray hits an inner shell electron of the atom which results in the ejection of the electron from the atom. A further outer shell electron fills the open space and fluorescence radiation is emitted. The emitted X-rays are gathered and differentiated based on their energies which are unique for each element [19]. A fluorescence energy spectrum is generated and processed for qualitative or quantitative analysis using the EDXRF instrumentation technique.

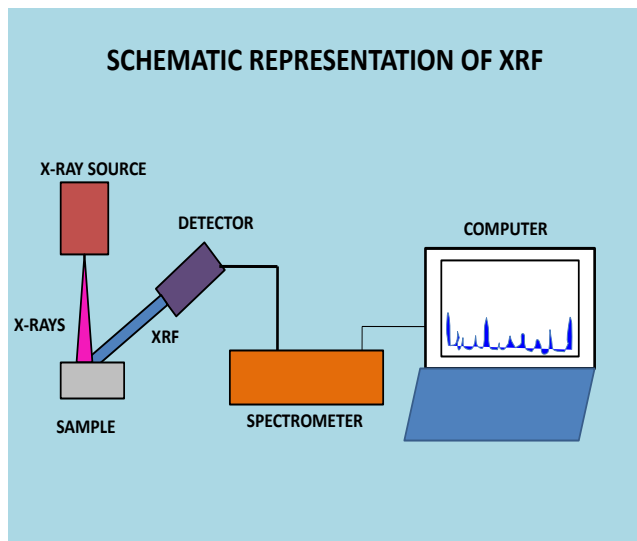


Figure 6: Outline of X-ray Fluorescence

Observations of glass examination:

Glass pieces were found in the left and right shoe of the culprit 1 and tallied with glass pieces found in the car of victim and glass pieces collected by medical officer which were found on weapon seized from culprit on the basis of hue, physical properties and spectro chemical composition.

Observations of chili powder examination:

Chili powder was detected on the window glass pieces inside the car of victim and in the shoes of culprit 1.

Knife and knife cover examination:

Two knives of different lengths were seized from culprit 2 during investigation. A cloth knife cover was found near the car of the victim. Probability of the knife cover to be of one of the knives could not be ruled out. Careful examination of the cover and both the knives was done to know whether the cloth knife cover belongs to knife 1 or knife 2.



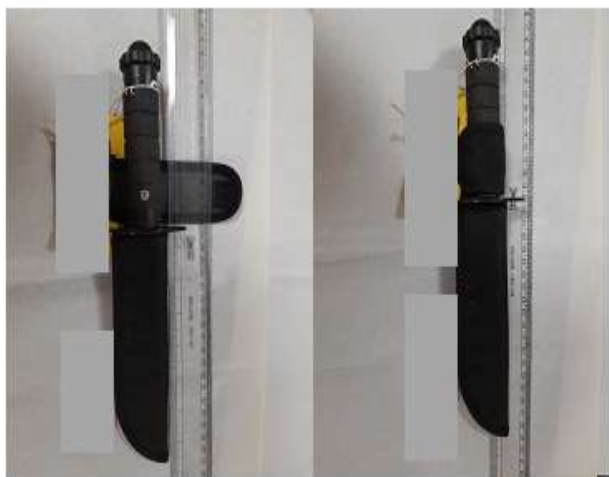
Figure 7: knife cover recovered from crime scene and knife 1 and knife 2 seized from culprit 2.

Alignment of knife 1 with the cloth knife cover showed that some of the part of blade do not fit in the cover and comes out of it. Also the buckle strip provided in the cover does not secure the knife properly. Therefore the alignment shows that the knife 1 does not fit in the cloth knife cover.



Figure 8: Knife 1 in the knife cover

Alignment of knife 2 with the cloth knife cover showed that entire knife blade fits in the cover. The buckle strip provided in the cover secures the knife properly in place. Therefore the alignment shows that the knife 2 fits in the cloth knife cover.



ALIGNMENT OF KNIFE 2 WITH KNIFE COVER

Figure 9: Knife 2 in the knife cover

Observations of knife and knife cover comparison:
Knife cover found on the crime scene showed characteristic physical matching fit with knife 2 seized from culprit 2.

Ballistics Division:

Magazine from country made pistol from crime scene and country made pistol without magazine seized from culprit 3 were sent for examination. As the magazine was recovered from crime scene and the pistol from the culprit, the intended use of pistol in the assault needed to be examined. Examination of evidence from firearms that may have been used in a crime constitutes Forensic Ballistics. When a bullet is fired from a gun, the gun leaves microscopic marks on the bullet and cartridge case. These marks are like ballistic fingerprints. When a bullet is recovered from the crime scene and the suspect's gun is seized, then a test fire is carried out with the suspect's gun. A comparison is made between marks on the crime scene bullet and the marks on the test-fired bullet. Assessment of the similarity of two sets of marks is done to determine if the bullet is likely to have been fired from the same gun or a different gun.

Gunshot Residue or cartridge discharge residue or gunfire residue or firearm discharge residue consists of all of the particles that are expelled from the muzzle of a gun following the discharge of a bullet. That mainly comprise unburned or partially burned gunpowder particles, soot, nitrate, and nitrites from the combustion of the powder, particles of primer (oxides of lead, antimony and barium)

and particles of the bullet or the bullet jacket that are vaporized when a firearm is discharged [20]. Organic compounds mainly originate from propellant and firearm lubricants, taking the form of unburned and partially burned gunpowder particles, some products of their transformation, and hydrocarbons. Inorganic residues such as nitrates, nitrites, and metallic particles originate from the primer and propellant as well as the cartridge case, the projectile jacket or its core and from the weapon barrel itself [21].



Figure 10: Distribution of Gun Shot Residue

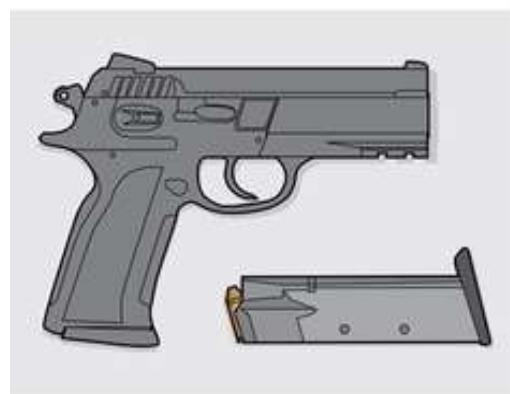


Figure 11: Image of Pistol and Magazine

Observations of magazine and pistol examination:
Magazine from crime scene was found to be magazine of country made pistol and it readily sat in the butt stock cavity of the country made pistol.
Chemical analysis of barrel washings of the pistol revealed residue of fired ammunition- nitrite, showing that the pistol

was used for firing prior to its receipt in the forensic laboratory.

Cyber Division:

CCTV footage of the crime location and nearby location along with reference photographs of the culprits were sent for identification of culprits.

Forensic video analysis involves extraction of footage with target for further analysis. Analysis depends on the type of camera, position of camera and its configuration [22]. Identification, preservation, analysis, documentation and presentation are the steps involved in Digital forensics. It helps to recover, analyze, and preserve computer and related materials in such a manner that it helps the investigation agency to present them as evidence in a court of law. It aids in determining the identity of the main culprit and motive behind the crime.

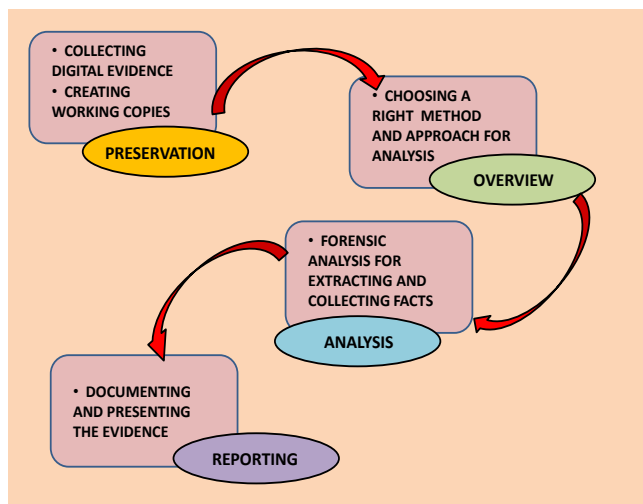


Figure 12: Schematic workflow of Digital forensics

Data related to video recordings are stored on the hard disk only. DVR machines can only store logs. Image Master Solo4/TD2 Imager/logicube are the imaging tools used for creating a mirror image of the hard disk provided. The mirror image is then installed in the DVR machine and the targeted video is searched. Back up of the targeted video is taken on a pen drive or hard disk for further analysis [23]. All the log records captured in the DVR machine are saved to keep a record of what activities were logged in the DVR machine viz. switch on/ off timing, date and time changed, etc. The hash value of the targeted video in the pen drive is calculated. The hash value can be described as the file fingerprint. Just like DNA is used to authenticate physical evidence at a crime scene, hash values are used to authenticate electronic evidence. The contents of a file are processed through a cryptographic algorithm, and a unique numerical value – the hash value - is produced that identifies the contents of the file. The hash value is generated at the time of imaging the evidence during data

acquisition [24]. Hash values are important when the evidence is admitted into court because altering even the smallest bit of data will generate a completely new hash value. AMPED Five tool is used for video framing, enhancing the video or particular frame of the video. Reference photograph of a person is matched with the person in the video recording by video analysis or frame by frame analysis.

Observations of CCTV footage examination:

- One culprit was detected in the video at crime location wearing a mask and holding a weapon.
- Two culprits were detected waiting at nearby square for victim to come and later five culprits came at the same square.
- Five culprits seen at various locations near crime spot one day before crime and also on the day of crime.
- On the day of crime, two culprits placed their vehicle in front of victim's car to obstruct his way. Three culprits came from behind with weapons and broke window glass of victim's car and attacked victim.

II. CONCLUSION:

The criminal investigation is a complex procedure requiring a multi-faceted, problem-solving attitude. Whenever a crime is committed, its detection involves three stages viz. discovery of the committed crime, suspect identification and sufficient evidence collection for the court of law. The evidence found at the crime scene a) locates the criminal, b) imparts one or more links to the crime, c) fortifies weak linkages and d) establishes accuracy or otherwise of the statements thus proving the innocence or guilt of a person. Forensic evidence assists in solving most brutal cases as well as completely nonviolent criminal cases such as fraud, hacking, drug possession and/or consumption, etc. It is the foundation on which prosecution and defense can build their structures. Therefore, utmost care should be taken during the collection, preservation and analysis of these evidences. The present case describes how evidences collected at the crime scene and from the culprits provided results through biological, chemical, physical and digital analysis. A common thing happening in the criminal justice system is when the witnesses turn hostile. A false statement from the witness and the whole case of the prosecution is shattered. The multidisciplinary forensic analysis involved in this case has relieved the prosecution from the dependency on eyewitnesses. Proper collection and preservation of evidence leads to precise forensic analysis and helps the judiciary with unbiased scientific results. Thus, forensic science with all its disciplines has become an indispensable tool in the hands of the judiciary for criminal justice administration.



Competing interests: The authors declare that they have no competing interests.

Acknowledgements: We are thankful to our Director General (Legal and Technical), Home Department, Govt. of Maharashtra and Director, Directorate of Forensic Science Laboratory, Mumbai for their guidance and encouragement all the time.

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