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BLOODSTAIN PATTERN ANALYSIS - AN OVERVIEW

S. Asadulla*[1], S. Arshiya Banu[2] and P. Salomi[3]

[1]M.Pharm, Department of Pharmaceutical Analysis & Quality Assurance, Sultan Ul Uloom College of Pharmacy, Hyderabad, A.P-500034.

[2]Pharm.D(PB), 1st Year, Department of Pharmacy Practice, P. Rami Reddy Memorial College of Pharmacy, Kadapa, A.P – 516003.

[3]Assistant Professor, Department of Pharmaceutical Analysis, P. Rami Reddy Memorial College of Pharmacy, Kadapa, A.P – 516003.

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***Correspondence for Author**
S. Asadulla
M.Pharm, Department of Pharmaceutical Analysis & Quality Assurance, Sultan Ul Uloom College of Pharmacy, Hyderabad, A.P-500034.

ABSTRACT

Bloodstain pattern analysis is one of the most challenging area in Forensic science. The main objective of this is to get information about the crime scene under investigation. It is a powerful tool used in solving violent crimes and must be performed by well-trained individuals. It is one of the most effective methods of reconstructing the crime scene available to forensic science. The Methodology includes DNA sample processes involving biology, technology and genetics. In Biology, collection of biological material from a crime scene and Isolation of DNA from its cells is carried out and the specific regions are copied by PCR (Polymerase chain reaction). Technology includes separation and detection of DNA by using various techniques like Slab gel electrophoresis and Capillary electrophoresis etc, and Genetics include comparison of genotypes. In this case the combination of individual STR genotypes (crime scene evidence) is compared with that of reference samples such as victims or suspect and as so in paternity investigations, a child's genotype would be compared to his or her mother's and the alleged father(s) under investigation. Finally a case report or paternity test result is generated. This report typically includes the random match probability to denote whether it is not matched or matched. The results of Bloodstain pattern analysis involves DNA analysis through which suspect can be caught with witness. The technique mainly used for DNA Fragmentation is Slab gel electrophoresis and Capillary gel electrophoresis and the types of DNA evidence

analysis includes Polymerase chain reaction(PCR), Restriction fragment length polymorphism(RFLP), Short Tandem Repeats(STR) and Mitochondrial DNA analysis.

KEY WORDS: Bloodstains, DNA analysis, Crime investigation, Forensic Science.

INTRODUCTION

There are hundreds of methods and analytical techniques used in the forensic laboratory and in that one of the important technique used by crime scene investigators is the analysis of stains left by bloodshed at a scene. So, bloodstain pattern analysis is regarded as a powerful tool in crime scene investigations. The science of bloodstain pattern analysis applies scientific knowledge from other fields to solve practical problems and crime scene. This analysis follows the scientific disciplines of biology, chemistry, mathematics and physics. If an analyst follows a scientific process, this applied science can produce strong, solid evidence, making it an effective tool for investigators.[1]

Crime scene photography is one of the way of documenting the crime scene in bloodstain pattern analysis. Special attention must be given to that bloodstains when there is a bloodletting scene. The current means of documenting the scene include 35 mm (B&W, color, and specialty film), digital cameras, and video (Hi-8, DV, and other formats). Each method has its pros and cons.[2]

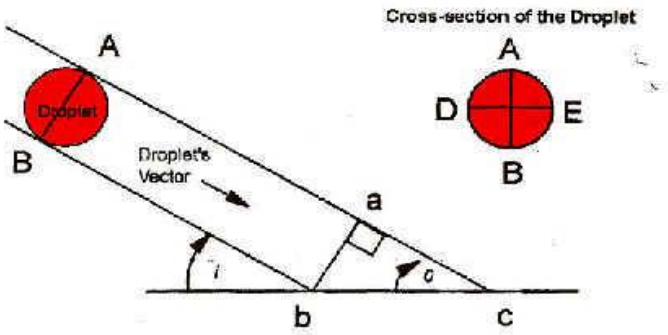
The success or failure of any criminal investigation often depends on the recognition of physical evidence left at a crime scene like bloodshed which gives a wealth of information by proper analysis. For the reconstruction of the events that occurred bloodstain pattern, size, shape, and the location of such stains may be very useful. Examination of these factors is carried out, in order to provide an interpretation of the physical events which gave rise to their origin. The investigative information obtained in bloodstain pattern analysis is highly accurate. Bloodstain pattern analysis stands alone to define truth that had occurred exactly in the crime scene.

In bloodstain pattern analysis, documentation is also done by examining the blood physical properties like gravity, viscosity and other factors like directionality and angle of impact.[3]

DIRECTIONALITY OF BLOODSTAINS AND ANGLE OF IMPACT

Directionality: When a droplet of blood strikes a surface perpendicular(90^{o}) the resulting bloodstain will be circular. In this case length and width of the stain will be equal. Respectively the length and the width will not be equal in the blood that strikes a surface at an angle less than 90 degrees.

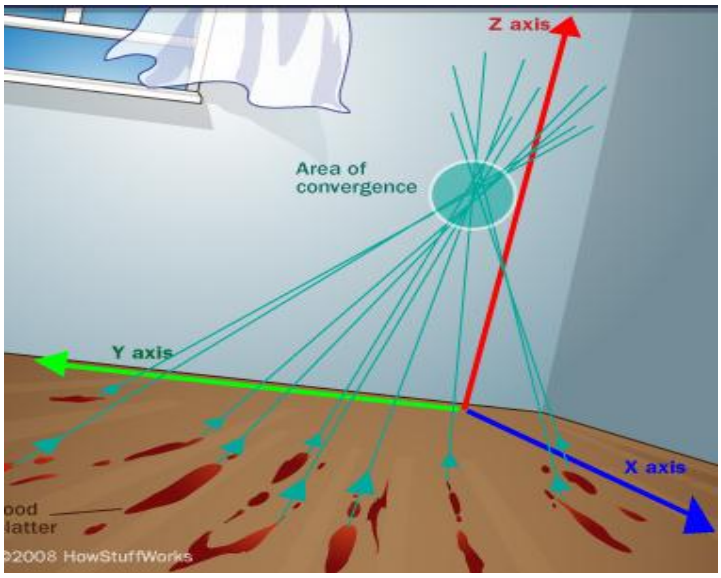
Angle of impact is the acute angle formed between the direction of the blood drop and the plane of the surface it strikes**.**



POINT OF CONVERGENCE AND ITS ORIGIN DETERMINATION

Point of Convergence is a common point on a two dimensional surface, over which the directionality of several bloodstains can be retraced.

- Once the directionality of a group of stains has been determined, it's become easy to determine a two dimensional point or area for the group of stains.
- The point of convergence can be determined, by drawing a line through the long axis of a group of blood stains and where the lines of the group of stains intersect one another the convergence point can be established.



Before documenting and collecting blood evidence, a crime scene investigator he or she must recognize the value of this evidence and how it fits in the overall events associated with the crime. The most common applications of blood evidence are:

1. Finding blood with the victim's genetic markers (ABO blood type, DNA profile, etc.) on the suspect, on something in the suspect's possession, or something associated with the suspect (such as the suspect's fingerprints).
2. Finding blood with the suspect's genetic markers on the victim, on something in the victim's possession, or something associated with the victim.
3. Investigative information determined from blood spatter and/or blood location
4. Impact spatters tells the time and space of the single event whereas the arterial damage not only describes the time but also tells how the assailant has used the weapon and how the victim moved after injury.[4]

BLOOD DETECTION METHODS

Some of the blood detection methods used in bloodstain pattern analysis are:

Physical Examination

Light Source

The Investigators has to examine the crime scene and look for areas that may contain blood. They may also use a high-intensity light or UV lights that helps in finding traces of blood as well as other bodily fluids that are not visible under normal lighting conditions.

Presumptive tests

Blood Reagent Tests

These tests are used to detect blood at crime scenes based upon the properties of haemoglobin present in the blood.[5] Further tests at the crime lab can determine if it is human blood or not.

Kastle-meyer test

In this test Phenolphthalein is used as a chemical, that is still utilized today and is usually referred to as the Kastle-Meyer test and produces a pink color when reacted with hemoglobin.

Hemastix

HemaStix is a strip that has been coated with tetramethylbenzidine (tmb) and will produce a green or blue-green color with the presence of haemoglobin.

Luminol

Luminol is used by crime scene investigators to locate the traces of blood, even if it has been cleaned or removed. Investigators spray this chemical solution throughout the area under investigation and look for reactions with the iron present in the blood, which causes a blue luminescence. But one of the problem with this test is, it reacts with metals, paints, cleaning products and plant materials.

Fluorescein

It is also capable of detecting latent or old blood. However, a special light and goggles are used to detect any illuminated areas, which appear greenish-white if blood is present. It may also react to many of the same things as luminol (copper and bleach).[6]

LCV or Leuco Crystal Violet

It is one type of chemical process which is used for blood enhancement, to make the blood evidence more visible so it can be photographed and analyzed easily.

By collecting the samples or biological material from a crime scene or paternity investigation, the DNA analysis is carried out. The DNA is first extracted from its biological source material and then measured to evaluate the quantity of DNA recovered.

Extraction of DNA

Before profiling the DNA, the DNA can initially be extracted from the samples.

The basic method for DNA extraction used at VFSC (Victoria Forensic Science Centre) is referred to as 'Chelex DNA extraction'. In this method the sample is boiled with the Chelex which breaks down the proteins and other cellular material and it prevents the DNA being broken down. The DNA is then separated from the remains of the proteins and other cellular material.

Generally VFSC analyses blood, hair and semen. Hair and spermatozoa have particularly tough cell membranes, which require the addition of other chemicals to break them down. Once the DNA is extracted the amount of DNA present in the sample is determined.

DNA Profiling Techniques

After isolating the DNA from its cells, specific regions are copied with any of the following techniques.DNA provides valuable information about the sample taken. [7]

TECHNIQUES USED IN DNA EVIDENCE ANALYSIS

Restriction Fragment Length Polymorphism (RFLP)

The RFLP method was the first DNA profiling technique used in casework in Victoria in 1989. In RFLP analysis, by using restriction enzymes the DNA sample is broken into pieces and (digested) and the resulting restriction fragments are separated according to their lengths by gel electrophoresis and transferred to a membrane via the Southern blot procedure[8] to which is applied a 'probe', a radioactive piece of DNA that specifically attaches to certain fragments depending on their type . The position of the probe is found by placing the membrane next to a sheet of radioactive film, resulting in the DNA fragments appearing as 'bands'.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR), uses an enzyme (polymerase) to replicate DNA regions of interest in a test tube by repeating the copying process, a small number of DNA molecules can be reliably increased up to billions within several hours[9] and thus permits very minute amounts of DNA into lot of copies which is used for examining. The resulting PCR products are then separated and detected. It is used in the evaluation of unsolved cases in which

evidence might have been improperly collected or stored and its main advantage is that it is able to be used with smaller amount of DNA or damaged DNA.

Short Tandem Repeat (STR) Analysis

STR DNA analysis evaluates specific regions (loci) that are found on nuclear DNA. Multiple STR regions can be examined simultaneously to increase the informativeness of the DNA test. These variable STR regions that are analyzed for forensic testing intensifies the discrimination between one DNA profile and another. For example, the likelihood that any two individuals (except identical twins) will have the same 13-loci DNA profile can be as high as 1 in 1 billion or greater.

The Federal Bureau of Investigation (FBI) has chosen 13 specific STR loci to serve as the standard for CODIS. The purpose of establishing a core set of STR loci is to ensure that all forensic laboratories can establish uniform DNA databases and, more importantly, share valuable forensic information. If the forensic or convicted offender CODIS index is to be used in the investigative stages of unsolved cases, DNA profiles must be generated by using STR technology and the specific 13 core STR loci selected by the FBI.

Mitochondrial Analysis

Mitochondrial DNA (mtDNA) technology analyzes the DNA found in a different part of the cell, the mitochondrion.[10] While RFLP and PCR techniques analyze DNA extracted from the nucleus of a cell. This analysis allows forensic laboratories to develop DNA profiles from evidence that may not be suitable for RFLP or STR analysis. Old remains and evidence lacking nucleated cells — such as hair shafts, bones, and teeth that are unamenable to STR and RFLP testing may yield results if mtDNA analysis is performed. For this reason, mtDNA testing can be very valuable to the investigation of an unsolved case.

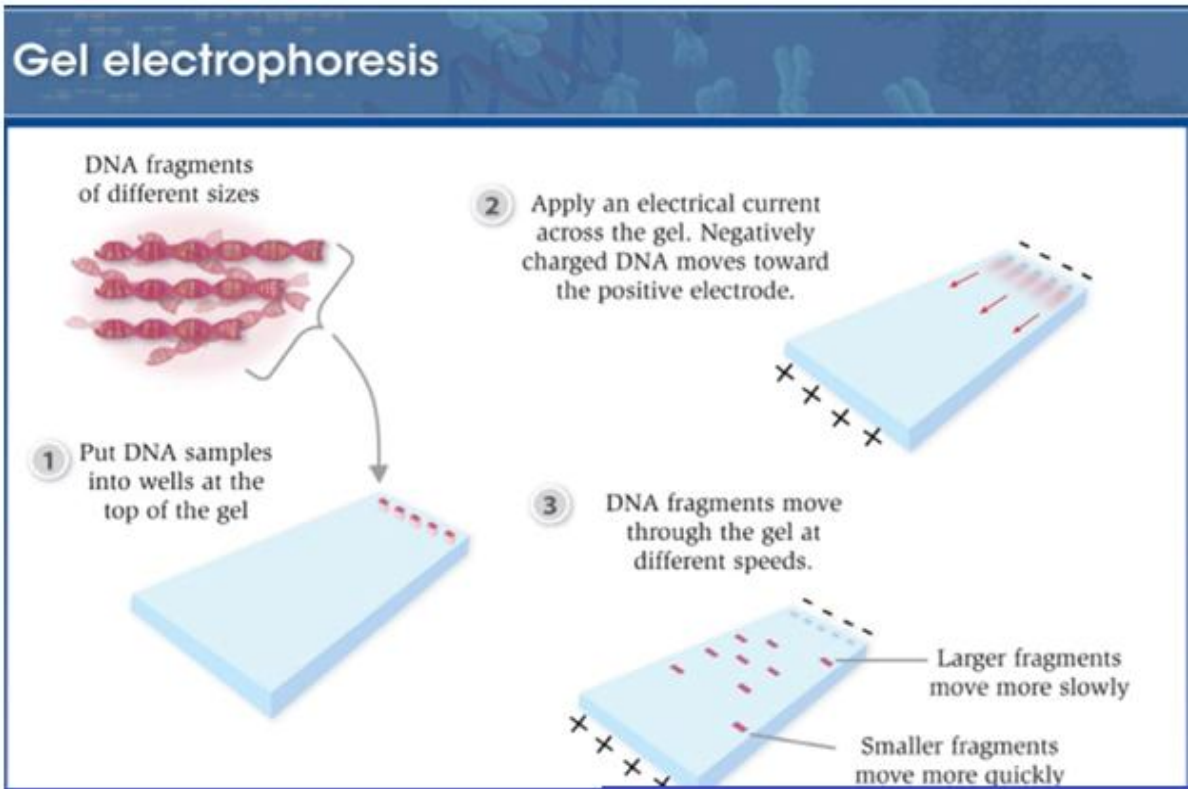
Another technique known as Y- chromosome is also used in DNA analysis. Y-chromosome markers target only the male fraction of a biological sample. Therefore, this technique can be very valuable if the laboratory detects complex mixtures (multiple male contributors) within a biological evidence sample. Because the Y chromosome is transmitted directly from a father to all of his sons, it can also be used to trace family relationships among males.

METHODS FOR DNA ANALYSIS

The separation methods used today include slab gel and capillary electrophoresis (CE).

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, NA and proteins) and their fragments, based on their size and charge. In this nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving. Proteins are separated by charge in agarose because the pores of the gel are too large to sieve it.

Slab Gel Electrophoresis



Slab Gel Electrophoresis is a process which enables the sorting of molecules based on size. Using an electric field, molecules (such as DNA) can be made to move through a gel made of agar or polyacrylamide. In slab gel electrophoresis, the electric field consists of a negative charge at one end which pushes the molecules through the gel, and a positive charge at the other end that pulls the molecules through the gel. The molecules being sorted are dispensed into a well in the gel material, these gel is placed in an electrophoresis chamber, which is then connected to a power source.

When the electric current is applied, the larger molecules move more slowly through the gel while the smaller molecules move faster. The different sized molecules form distinct bands

on the gel.[11] After the completion of electrophoresis the molecules in the gel can be stained to make them visible. DNA can be visualised using ethiumbromide or fluorescent under ultravoilet light. The distance the DNA copies have travelled through the gel is compared to the distance that known standards have travelled.

Capillary Gel Electrophoresis

Capillary gel electrophoresis is generally performed in a porous gel matrix with buffer that fills the pores of gel. This type of media provides molecular sieving action that leads to migration of analyte species to various extent depending on pore size of polymer and size of analyte.[12]

This sieving action is helpful in separating macro molecules such as proteins DNA fragments, oligonucleotides that have similar charge but differ in size. DNA from the sample is extracted same as gel electrophoresis method. Thus DNA fragment with required genetic code is obtained.

The detection of DNA sequence can be determined by capillary electrophoresis is as follows: First DNA is fragmented and the fluorescent dyes are attached to various DNA fragments. Now the sample may contain different sized fragments with fluorescent dyes. When laser rays are passed, fluorescent dye which is attached to the fragments undergo excitation and emit radiation. This emitted radiation is sent to detector. Upon reaching the detector, the DNA sequence can be determined by dye colour sequence of eluting fragments.

Low molecular fragments move faster to detector than high molecular fragments. Hence it is concluded that first reached fragment has low molecular weight and dye colour sequence tells as about the DNA fragment sequence.

Finally a case report or paternity test result is generated. With paternity investigations, a child's genotype would be compared to his or her mother's and the alleged father(s) under investigation. If there is not a match between the questioned sample and the known sample, then the samples may be considered to have originated from different sources. The term used for failure to match between two DNA profiles is 'exclusion.'

If a match or 'inclusion' results, then a comparison of the DNA profile is made to a population database, which is a collection of DNA profiles obtained from unrelated individuals of a particular ethnic group.

CONCLUSION

Bloodstain pattern analysis is an important tool in the field of Forensic Science. As it is involved with gathering and examining the criminal evidences. According to bloodstain pattern analysis blood found at the crime scene plays very prominent role in Forensic Science, as it helps in sorting out of criminal cases and enforces law in the society. The main function of bloodstain pattern analysis is to support the witness statements and laboratory and post-mortem findings.

Physical properties of blood like surface tension, gravity, viscosity and other factors like blood stains, their position and directionality , angle of impact of striking bloodstains makes bloodstain pattern analysis strong and solid evidence. Thus making it different from other techniques such as finger print technique, chemical analysis etc.

The main principle behind bloodstain pattern analysis is DNA analysis. The DNA is unique component in every person. So, blood found at the crime scene is analysed and DNA is detected. By comparing the suspect's and sample DNA, suspect can be caught with witness. There are various techniques of analyzing DNA like PCR, RFLP, STR, by slab gel electrophoresis and capillary gel electrophoresis.

REFERENCES

1. Stuart H. James, Paul E. Kish, T. Paulette Sutton., Principles of Bloodstain Pattern Analysis: Theory and Practice**,** 2005; 2.
2. Lytle LT, Hedgecock DG., Chemiluminescence in the visualization of forensic bloodstains. J Forensic Sci. 1978; 23: 550–5.
3. Tom Bevel, Ross M. Gardner**.,** Bloodstain pattern analysis with an introduction to crimescene reconstruction, 3rd edition, 2008; 1-2.
4. Anita Y. Wonder., Bloodstain Pattern Evidence: Objective Approaches and Case Applications, 2007; 8.
5. Castello, Ponce and Fernando A. Verdu Psul "Critical Revision of Presumptive Tests for Bloodstains", Forensic Science Communications, jul 1999; 1(2): 1-151.
6. Cheesman R. "Direct Sensitivity Comparison of the Fluorescin and Luminol Bloodstain Enhancement Techniques', Journal of Forensic Identification, (1999).

7. Misawa S Department of Legal Medicine, University of Tsukuba. Rinsho Byori. The Japanese Journal of Clinical Pathology [1994; 42(6): 636-642] Type: Journal Article, Review, English Abstract (lang: jpn)
8. Hochmeister MN, Budowle B, Baechtel FS., Department of Forensic Medicine, Bern, Switzerland. Journal of Forensic Sciences [1991; 36(3): 656-661].
9. Bartlett, J. M. S.; Stirling, D. (2003). "A Short History of the Polymerase Chain Reaction". PCR Protocols. Methods in Molecular Biology 226 (2nd ed.). pp. 3–6.doi:10.1385/1-59259-384-4:3. ISBN 1-59259-384-4.
10. Wayne Petherick, Brent E. Turvey, Claire E. Ferguson., Forensic Criminology.
11. Wraxall, B.G.D.; Culliford, B.J. (1968). "A thin-layer starch gel method for enzyme typing of bloodstains". J. Forensic Sci. Soc. 8 (2): 81–82. doi:10.1016/S0015-7368(68)70449-7.PMID 5738223.
12. Butler, John (2004). "Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis" (PDF). Electrophoresis 25: 1397–1412.doi:10.1002/elps.200305822. PMID 15188225. Retrieved 2014-04-09.