10.5005/jp-journals-10037-1135

REVIEW ARTICLE

Recent Advancements in Human Blood Identification in Forensics

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ABSTRACT

Context: Crime scene investigation is one of the mainstream branches in forensics. Body fluids like blood, saliva, reproductive fluids, etc., and identification, collection, storage, and interpretation of these samples are of great importance in solving a case. This review article throws light into various techniques for analysis of these samples emphasizing on genetic material identification.

Objectives: This review elaborates on various procedures and techniques involved in forensics for the identification and analysis of blood samples from a crime scene.

Materials and methods: Data were obtained and analyzed from electronic database searches of relevant published literatures from PubMed and Google Scholar.

Conclusion: The advent of advanced techniques in the analysis of genetic materials from blood samples like short tandem repeat (STR) recognition, single-nucleotide polymorphism (SNP) analysis, mitochondrial deoxyribonucleic acid (DNA) analysis, microfluid system, and nanotechnology helps the scientists in forensic investigations.

Keywords: Forensics, Genetic analysis of blood sample, Human blood identification.

How to cite this article: Jithin P, Kadar N, Mohan A, Pynadath MK, Mohammed PKW, Kalliath C. Recent Advancements in Human Blood Identification in Forensics. Oral Maxillofac Pathol J 2018;9(2):79-84.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Biological fluids obtained from a crime scene contain genetic materials that may help in identifying a victim or a suspect, as well as in absolving an innocent accused.

Usually it is difficult to detect as well as collect such fluids from the crime scenes. Specialized techniques using alternate light sources for the detection of stains made by those biological fluids are necessary in this scenario. The specimens thus collected contain genetic materials like DNA which may be either intact or damaged and specialized techniques are used for the amplification and identification of such genetic materials which ultimately help in recreating the crime scene and identifying the suspect or victim.

Blood is a common and important finding in crime scenes. Blood may be either in the liquid form or dried state as well as stains on the objects. It may not be visible to naked eyes and requires specific identification techniques. Biological fluids often require use of a particular type of light source or chemical treatment to reveal their presence. Methods used to achieve this goal are called "presumptive tests."[1] Such methods are by no means conclusive by themselves, and further analysis remains essential.

The strength of DNA as a forensic tool is that it can be elicited and studied months or even years after it is partially degraded. Time, temperature, humidity, radiations, and chemicals affect the DNA integrity.

Blood Detection

Locating, investigating, and interpreting bloodstains on various surfaces help in recreating the crime scene. The forensic investigations that deal with this is known as bloodstain pattern analysis (BPA) founded in 1983.[2] The BPA involves gathering, classification, and interpretation of the bloodstain patterns found in crime scenes. Bloodstain patterns can be categorized as: Dripped and splashed blood, impact patterns, projected blood, expirated bloodstains, transferred bloodstains, and castoff stains.

In some cases, bloodstain can be detected, whereas in some cases, it may not be evident because of lack of contrast and/or absorption of the fluid. Alternative light sources (ALSs) are used to visualize the fluorescence with improved contrast. Bloodstains have an absorption rate of 300 to 900 nm which covers wave lengths from ultraviolet to infrared.

This helps in utilization of ultraviolet rays as alternate light source for visualization. Crimelite, Crimescope, Mini Crime scope, TraceEr Laser, Ultra Lite Als, etc., are the commercially available ALS. Modern devices help detect both red and brown bloodstains.

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If these methods did not help in identifying the bloodstains or the scene has been washed to remove stains, alternate chemical methods to visualize the bloodstains are required. Chemiluminescent reagents are used for conducting presumptive tests; and they reveal latent bloodstains in crime scenes, which are not visible to naked eye.

Several chemical agents like ortho-toluidine, benzidine, and tetramethylbenzidine help in visualization, but cause DNA damage, whereas others like amido black and luminol do not cause DNA damage. Amido black is an organic dye which stains the hemoglobin and produces a dark blue-black image. It is used for detecting bloodstained finger prints. It can be used in porous as well as nonporous surfaces and does not cause DNA damage.

Chemical agents like Acid Yellow-7 are used for the uncovering of blood-stained finger as well as shoeprints. Prints become yellow after treatment and fluoresce under blue or blue-green light (385–509 nm).[3-14] If the bloodstain is left for a long time, it changes its color from red to dark brown.

In such cases, luminol is frequently used as the reagent of choice if the crime scene has been cleaned. Luminol reacts with iron in the hemoglobin and creates a blue chemiluminescence in the dark.[15,16] Other chemical agents like Kastle-Meyer test, Sangur stick test, Hemastix test, Leucomalachite green test, Leucocrystal violet test, etc., are also used.

These tests are based on the fact that the heme groups of hemoglobin possess a peroxidase-like activity which catalyzes the breakdown of hydrogen peroxide. The oxidizing derivatives formed can then react with a variety of substrates and produce a visible color change.

Samples of wet bloodstains must be collected using a swab, and sealed in an airtight container for comprehensive examination. Dried bloodstains should be scraped onto a sheet of clean paper, or into a paper bag.[2]

Estimation of Age of the Blood Sample

Old blood samples are more prone to errors while detecting various proteins as well as genetic materials from the sample. Various nongenomic and genomic methods are used for the age estimation of the sample. Nongenomic methods like high-performance liquid chromatography, reflectance spectroscopy, oxygen electrodes (determine the amount of HbO_2), electron paramagnetic resonance, atomic force microscopy (determines the elasticity of extracorporeal red blood cells) are used for age estimation of the sample.

Genomic methods like real-time polymerase chain reaction (RT-PCR), STR segment analysis, etc., are used for age estimation of the sample.[17]

ABO Blood Group Recognition

ABO blood grouping was one of the most commonly used tests during older time, but it has very limited application. It can eliminate a possibility, whereas it cannot confirm a possibility in the crime scenario.

A recent study showed interest of an indirect competitive enzyme-linked immunosorbent assay (ELISA) technique, as conventional ELISA needs immobilization of ABO blood group antigens located on the membrane surface, which are difficult to extract from dried stains, except if one uses solubilization with detergents or organic solvents.[18-22]

Genetic Material Detection from Blood Samples

Since the commencement of forensic DNA analysis in the 1980s, it has gone through a number of stages of development.

First Generation of DNA Analysis

Restriction fragment length polymorphism (RFLP) profiling is no longer used by the forensic group of people, as it requires relatively great amount of DNA and ruined samples could not be analyzed with precision. The second generation of DNA analysis was based on PCR and mainly consists of dot-blot techniques. However, it is not suitable in the analysis of longer strands of DNA.

The third generation of DNA analysis is STR analysis. More efficient, faster, and cheaper DNA analysis techniques are constantly being refined. The RFLP profiling may be considered as the first generation of DNA analysis methods.

The RFLP is a procedure that exploits variations in homologous DNA sequences. It refers to a dissimilarity between samples of homologous DNA molecules from contrary locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated.

In the RFLP analysis, the DNA sample is broken and digested by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. Polymerized chain reaction combined with RFLP is used nowadays with improved accuracy.

Polymerase chain reaction is a technique used to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.[21-24] Advantages of the PCR-RFLP technique included low expensiveness and unnecessity of advanced technology.

Repetitive DNA regions, which are located outside the coding regions of DNA, are used for further analysis of DNA. They are different for each individual and can

be used for personal identification as well as a group of people, such as a group of family members. Developed by Mullis in 1983, PCR continues to be a valuable tool in forensic DNA analysis.

Polymerase chain reaction is able to replicate specific nucleotide sequences from low levels of DNA or degraded DNA. Amplification involves the addition of DNA primers, nucleotides, and DNA polymerases, which are then taken through a series of temperature changes. The detection of DNA is further confirmed through the use of fluorescence, which uses fluorescent dyes that attach to PCR primers in the amplicons.[25-27] Forensic scientists use the slot-blot hybridization approach to target the D17Z1 locus, a highly recurring alphoid primate-specific sequence for DNA quantification in forensic work.

The development of RT-PCR human DNA quantification kits has contributed to the popular use of RT-PCR in forensic genetics. Real-time PCR or quantitative PCR has been used to detect male DNA in sexual assault cases. A DNA quantification assay that uses Alu, a highly repetitive DNA sequence, has been able to detect down to 4 pg during DNA quantification which is highly specific and sensitive.[28] More advanced variations of PCR like digital PCR (dPCR) offer a higher degree of precision.

In combination with duplex reactions, where two targets are analyzed per reaction, dPCR can prove to be more precise measurement. Particularly in cell free DNA analysis, duplexing dPCR can reduce the number of individual PCR reactions. Other quantitative methods that are able to detect picogram levels of DNA include the hybridization method and the threshold method.

The hybridization method uses either radioactive isotopes or chemiluminescent compounds, whereas the threshold method is mediated by antibodies. Another variant called real-time degenerate oligonucleotide primed PCR (DOP-PCR) can amplify the whole genome regardless of DNA size. It is also independent of DNA sequence and can be used for many different species, giving it a universal property.[29]

Advancements in DNA Analysis

The STR analysis PCR technique can be used to amplify STR typing with highly polymorphic DNA sequences of repeating 2 to 7 base pairs. The amplification of STR, via PCR, starts with targeting loci by sequence-specific primers. Electrophoresis is used to separate the DNA fragments.

The STR markers in human identification need to exhibit the highest irregularity among individuals and are measured by the lengths of the diverse alleles. The STRs are generally categorized by the length of their repeat: mono-, di-, tri-, tetra-, penta- and hexa-nucleotides. Tetranucleotides are most often used in STR analysis because they have a smaller probability of stutter products, amplicons that are one repeat less than the true allele.

Higher molecular weight STR loci are difficult to amplify and result in a DNA profile that is incomplete. Moving the PCR primers closer to the STR region has been able to beat some of these difficulties. These reduced size STR amplicons are often referred to as "miniSTR" assays and are able to obtain information from degraded DNA.[31]

Single-nucleotide Polymorphism Analysis

The SNPs offer a benefit over STRs due the fact that greatly degraded DNA fragments can be analyzed with SNPs.

The SNPs are base substitutions, insertions, or deletions and occur only at one position of a genome. It is the biallelic nature of SNPs that can aid in DNA profiling but also make them not as revealing per locus as STR and difficult in detection when working with DNA mixtures. The SNPs have a low mutation rate rendering them more established as genetic markers.

The ABO genotyping used along with SNP can be used to decide the ABH antigen expression that can be found on the surface of red blood cells. Variations of the SNP detection use four ABO loci and an amelogenin gender marker, so that individual identification and paternity testing can be done simultaneously. The DNA chips can also be used as a tool for analyzing SNPs.[33] Degraded DNA has been successfully amplified for STR genetic profiling using whole-genome amplification (WGA).

Among the variation of WGA, there are DOP-PCR, primer extension preamplification (PEP), multiple displacement amplification, blunt-end ligation-mediated WGA, rolling circle amplification (RCA), and restriction and circularization-aided RCA. The PEP is established to work well with highly degraded DNA or low copy number DNA.[34]

Mitochondrial DNA Analysis

Mitochondrial DNA (mtDNA) found in the mitochondria are tiny organelles in the cell, not associated with the nuclear chromosomes.

The mtDNA remains as a viable source because of its quantity. It is often used in degraded DNA due to the higher proportional amount of mtDNA to nuclear DNA and are less prone to degradation. The hypervariable regions of mtDNA are used for analysis.

Modified multiplex PCR systems are used to produce small overlapping amplicons that are used to determine the sequence of mtDNA. The mtDNA analysis and SNP-based screening methods provide a higher sensitivity.[35]

Deoxyribonucleic Acid Methylation Analysis

The DNA methylation appears to be the best suited for body fluid identification at this time, because of its high specificity and compatibility with current STR typing protocols. There are tissue-specific differential methylated regions that are seen in the genome of mammals.

The PCR methods that are methylation specific are combined with existing STR typing protocols. It helps in estimating the age of the individual. This assay uses a set panel of loci that are differentially methylated in between tissues to conclude the most probable source tissue of an unknown DNA sample.[30-35]

Microfluidic Systems for DNA Analysis

A microfluidic system is composed of two or more microdevices or chips that can perform a single processing step, such as microcapillary electrophoresis. The PCR microdevices are commonly made of glass or from polymeric substrates, such as polymethylmethacrylate.[34] The use of robotics is becoming more and more incorporated into the microfluidic systems.

The appeal is the fact that extraction and purification can be automated, reducing cost, time, and the chance of contamination. An automated DNA extraction platform which uses magnetic particle chemistry provides a robust and more cost-effective method of extracting DNA with less chance of contamination.

One technique used to integrate the extraction and purification in a microfluidic system is the use of surface-charge switchable magnetic particles, which help with the flow of nucleic acid through the system. Low-frequency electric fields can offer an alternative to the reagents that are commonly used for the fluorescent recognition and extraction step of microfluidic systems.[35,36] In three steps, thermal denaturation, annealing, and extension can occur, reducing reagents and chance of contamination.

Even in cases where most of the DNA samples are annihilated or when only limited amounts of DNA samples are available, improved multiplexes have been able to identify STR profiles. Other variations of the microfluidic system include the use of various enzymes. Inability to quantify DNA is one of the main drawbacks of a microdevice.

Nanotechnology for DNA Analysis

Nanoparticles have begun to be included into the process of PCR amplification due to their distinctive ability to create physical and chemical properties based on what may be on their surface. For example, gold nanoparticles can develop specificity and increase PCR efficiency.

Specificity of PCR is also enhanced by silver nanoparticles, carbon nanotubes, and nanometer-sized polymers. Recently, nanotechnology has been gaining popularity, as it can create devices that can assemble, manipulate, and analyze things at very small levels. Providing a system for DNA detection that is portable, quantitative, and available to the public is the goal of nanotechnology in the field of forensic science.[29-37]

Deoxyribonucleic Acid Databases

National DNA databases are maintained by the governments to store DNA profiles of the people based on PCR, and using STR analysis.

Their purpose is to search and match DNA profiles of potential criminal suspects. The Interpol DNA gateway contains DNA profiles, which are submitted by different member countries. They contain profiles created from samples which were collected from sites of natural disasters, crime scenes, unidentified bodies, and missing people.

It has more than 140,000 DNA profiles contributed from 69 member countries. The combined DNA index system (CODIS) loci CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 are now nationally and internationally recognized as the standard for human identification.[38] (CODIS is the United States national DNA database created and maintained by the Federal Bureau of Investigation.)

CONCLUSION

In this review, a brief analysis of blood as an investigative tool in various crime scenarios has been discussed. The methods of visualizing, identification, collection, storage, and analyzing the sample have evolved in recent years. Advanced techniques like STR recognition, SNP analysis, mtDNA analysis, and microfluid system have helped forensic scientists to solve the various cases. Nanotechnology has a new application in forensic science.

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