

Species Determination from Blood; An Advancement in Trend Towards the Investigation



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Abstract

Blood is the most prevailing and probably most important evidence in illuminating of convinced types of crime. It is frequently encountered in several forms i.e. liquid, blood stains, adulterated with foreign elements, infused with other biological evidences/ fluids or in various forms. The analysis of such evidences provides the link between the victim, suspect and even about the location. There are many obstacles standing in the way of accurately scrutinizing in the legal context that may be the species determination or the gender discrimination. The species determination from the blood stain is an immediate challenge for forensic science, veterinary purposes and wildlife conservancy. A rapid advancement and development has been done so far in this field which has facilitate and produce the investigation easier. Now a day, the modern methods used to identify the species of origin are restricted and destructive by nature. This review is study about the species determination from the blood stains, their advancement analytical important and role in an investigation.

Keywords: Blood; Species determination; Investigation; Biological fluids; Advancement

Introduction

Blood is the most frequently encountered biological evidence from the scene of occurrence. It is asset in various forms such as liquid blood stains, dried stains, adulterated along with other biological evidences, adulterated with physical evidences (soil, paint, etc.) and blood patterns. That provides the link between the victim, suspect and the place of commission and about the species of origin. For decades, crime scene technicians have been used chemical tests at crime scenes to identify suspected traces of blood or to locate blood evidences at scene of occurrence. In 1990, Paul Uhlenhuth invented the precipitin test which was based on the reaction of serum to human blood [1]. Following his steps in 1901, Karl Landsteiner invented the ABO blood groups which was later on divided into a series of antigen-antibody reactions in blood. The gradual changes in research in technology provided advance methods for blood as an evidence. Now a day, blood identification is done by following certain methods used for analysis which includes microscopic examination, chemical examination, immunological method, spectrophotometric analysis etc [2,3].

Along with these methods, Ring precipitin test took over the traditional methods and provide the information about to determination of the species from blood. Later on, a few of advance techniques emerged such as Antiglobulin consumption test (Hemagglutination inhibition test) that is based in the detection of globulins. Ouchterlony double diffusion assay methods were based in the agar gel plates that reacts with both antibodies as well as antigens [4]. For the qualitative and quantitative determination of blood crossed over electrophoresis technique is used. In this technique the influence of an electric field, the antigen and the antibody migrate towards each other and a precipitin is formed at the point of their interaction. The advancement continues and some of the highly specific anti haemoglobin precipitin sera have been used for the identification of human bloodstain in a single procedure. Other methods that include iso-enzymes method and rapid immunoassay are also used in which iso enzymes method of the same species in multiple molecule's existence determination also considered less sensitive method. Immunoassay test trips

for human blood involves the reaction of antigens in the extract with monoclonal antibodies within the test strip resulting in the extract antigen-antibody complex where it reacts with dye particles to create suitable visible reactions [5]. The blood group can be determined from the blood of human origin and can conclude the quantification of DNA by using some specific traditional techniques such as RFLP, PCR, PCR-RFLP, Species specific repeat polymerase chain reaction etc. that is carrying information about individual.

Some spectroscopic techniques such as Infrared Raman spectroscopy, spectrophotometry are also used to analyse multiple dry blood samples for forensic species identification purpose. At present, the spectrophotometric examination is based in the identification of haemoglobin and its derivatives through the specific absorption. The information is obtained in form of spectra due to the heme portion of haemoglobin molecule. The visible region of the spectrum of the heme derivative differs substantially from derivative to derivative, but all have in common a strong absorption band at 400-425nm [6]. Porphyrin compounds and their derivative from animal or vegetable sources may share spectral characteristics with haemoglobin, hematin or homochromogen. Therefore, the identity of bloodstain should never be inferred solely from a single absorption spectrum. Even in the spectroscopy technique, modernization has taken place which is based on the utilization of modern analytical methods based on the combination of Raman Spectroscopy and advanced statistics to analyse the composition of blood traces from several species. This principle is based on the components such as SFA, PCA, and other cross validation methods. The obtained 3D scores plot shows the significant separation between group of species.

In the third generation of DNA analysis or current method of choice is STR analysis which is more effective, faster and cheaper. The development in this method continues such as some of the recent progress made in the analysis is STPs, SNPs, low template DNA, and mitochondrial DNA & DNA methylation. Short tandem repeat analysis is used to amplify STR typing with highly polymorphic DNA sequence of repeating 2 to 7 base pairs of an individual which particularly focus on 5 to 10 alleles of a STRs in forensic profiling [7]. STR markers are identified by measuring the length of different alleles which are classified by mono, di, tri, tetra, penta and hexa nucleotides. Tetra nucleotides are most often used in STR analysis due to the fact they have a smaller probability of stutter products, amplicons that are one repeat less than the true allele.

Single Nucleotide Polymorphism (SNP) Analysis has made it possible to differentiate among the suspects/ individuals based on DNA that who is the actual person linked to the crime? Although the obtained information depends upon the quality of the evidences and it could show inability to create complete STR typing. Alternatives SNPs and insertion / deletion (indels) are often used to increase the PCR sensitivity of STRs, known as the degraded amplification. Analysis of Degraded or Low

Template (LT) DNA has been successfully amplified for STR genetic profiling using whole genome amplification (WGA) [8]. A few specific cases in which WGA can be used to amplify highly degraded DNA so that it can be further analysed in the PCR method. A variation of WGA that degenerate oligonucleotide-primed-PCR (DOP PCR), primer extension preamplification (PEP), multiple displacement amplification (MPA), blunt end ligation mediated WGA (BL-WGA), rolling circle amplification (RCA, and restriction and circularisation-aided rolling circle amplification (RCA-RCA), PEP is found to work well with highly degraded DNA or low copy number DNA.

Mitochondrial DNA (mtDNA) Analysis is done for the tiny organelles in the cell of mitochondria. The advance of these techniques takes over LT DNA that SNPs and WGA have made alternative source and methods continue to be looked into including mt-DNA and single cell analysis. Mt-DNA remains as a variable source because of its quantity. mt-DNA is often used in LT DNA due to higher proportional amount of mt-DNA to nuclear DNA and its ability to be less prone to degradation [9]. qPCR assays offer a high specificity that can be used in the analysis of mt-DNA. This assay can utilise a synthetic DNA that can further assure the quality of DNA. In addition, this technique can also indicate the presence of PCR inhibitors and indicate the need for further purification. Current techniques involved the use of mRNA, micro RNA, immune based assays and DNA methylation analysis. DNA Methylation Analysis is capable to determine the identity of suspect/ culprit and the type of source from which the DNA extracted including sperm, saliva, vaginal fluid and blood. DNA methylation appears the suitable technique for body fluid identification at present, due to its high specificity and compatibility with current STR typing protocols.

In some specific cases, there are time specific differential methylated region (t-DMRS) that occur in the genome of mammals. PCR methods that are methylation specific are capable to identify body fluid and could possibly be multiplexed with existing STR typing protocols. DNA methylation also has the potential to be used to help determine an estimated age.

Analysis of non-human DNA

A real time PCR genotyping. Involving the detection of a human specific nuclear gene target fork head box P2 (FOXP2) is used to distinguish between human and non-human DNA. FOXP2 can encode a transcription factor that accumulates amino acid changes in the human lineage, specifically involved in speech and language development in humans. The detection of FOXP2 can be used as a quantification method conducted further STR analysis preventing unneeded analysis of non human DNA [10]. Just as DNA chips have been combining chip technology and ABO genotyping can also be used to identify species.

Microfluidic Systems for DNA Analysis

Micro fluidic devices have become more popular since the invention of the 'Lab-on-the-chip'. Micro fluidic system is

composed of two or more micro devices which perform a single processing step that includes the micro capillary electrophoresis. The micro sizes allow for minimal reagent and the quantity of the sample. To avoid the chance of contamination, the system can be completely sealed. The micro device has potential to work efficient during the process to construct the type of valve, mixer or pump on system.

Utilization of Nanotechnology for DNA Analysis

With the early phase of advancement, Nano particles have begun to incorporate in the process of PCR amplification. Due to the unique ability to create physical and chemical properties and presence on various surfaces, this technology helps to determine the DNA. Gold nanoparticles (AuNPs) can improve specificity and increase PCR efficiency. Carbon nanotubes (CNTs), nanometre sized polymers and silver nano particles (AgNPs) have also been able to enhance specificity of PCR [11]. It is theorised that AuNPs are capable to work in the same fashion as SSBs; however, the actual mechanism is still under investigation. The nano particles has been becoming a field of interest due to their ability to analyse even the small quantity of the evidences.

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