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Blood stain identification and its DNA stability on different fabrics

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Abstract

In forensic sciences, blood samples that have been obtained from a place where a crime has taken place, is considered very imperative in analysis and criminal investigation. There are different methods by which forensic scientist can identify and extract genomic material from the source sample. Methods like Kastel-Mayer test, Benzidine test, Lumiscetin test, Fluorescence test etc are commonly used in forensic scientist to identify blood stain in laboratory or on a crime scene. Choice of method used by the forensic scientist generally depend upon the crime scene and evidences. In order to identify a real criminal DNA analysis is also very important. Isolation of DNA include lysis of cell, collection of DNA and concentration of it. There are PCR based techniques used now a day's which does not require cell lysis process. After isolation, gel electrophoresis was performed and separate bands of DNA are observed while visualizing gel under UV light. This paper present a review on different methods used in forensic analysis of blood stain.

Keywords: blood stain analysis, kastle-mayer test, DNA isolation

Introduction

In forensic sciences, blood samples that have been obtained from a place where a crime has taken place, is considered very imperative in analysis and criminal investigation. Blood essentially contains all the information (in the form of DNA as well as certain other chemical compounds) that is needed to narrow down possible suspects and also to determine the identity of the true criminal.

Blood is the major biological fluid present in the human body. It mostly performs the functions of transport of gases in dissolved form as well as transport of nutrients such as glucose and fatty acids ^[18]. Blood plays a very important role in the immune complex because it helps circulates the white blood cells that work against and destroy pathogens. In addition, it also performs the function of coagulation that help seal and prevent excessive bleeding. Human blood constitute about 55% of plasma and 45% of the cellular components. Of the cell components of the blood, red blood cells (RBCs) make up 45% of the total composition while thrombocytes make up 54%. RBCs are enucleated and they do not have any DNA at all. Thrombocytes are also devoid of any genetic material. The remaining 1-2% of leukocytes or white blood cells (WBCs) are the only group of cells that contain DNA and it is this DNA which is isolated during the DNA extraction process. A person can survive a fatal injury if the blood loss is not more than 1/4th of their total body blood. Any more blood loss can result in the person's death ^[17].

Analysis of blood stains is a sensitive procedure but it proves to be the most valuable source in solving a criminal investigation. It is an extremely specialized field of forensic science. At the time of searching clues and information regarding a crime case it can often proof very beneficial. There are different areas of interest in forensic science among them identification of blood stain is most important because after the analysis of blood cell we can get sample DNA through different DNA extraction techniques and can determine blood group also. Investigator may found reddish brown paints or any body fluid or a blood stain on a crime scene but it is important to identify the blood stain among them all because it is the Analysis of blood stain which provides a link between suspect, accused and a crime scene. There are two different conditions in which blood stains are found i.e dried or in liquid condition. Mostly dried blood samples are found over the clothes ^[1].

In our body, blood is a common fluid and hence it is most commonly present at the crime scene but there are various contaminations like microorganism, temperature, heat, moisture and other factors. Blood stain is affected by the concentration of acid and basic chemicals.

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There are different factors which can affect the biological evidence like heat, moisture, microorganisms, etc. They generally degrade the biological traces [3]. Beside this, chemicals can also deteriorate biological evidences. On the crime scenes stains of blood are found on clothes. According to the nature of fabrics, penetration of blood stain on clothes is affected. It's is texture of fabric which effect the absorption of blood stain and hence the degree of distortion. There are different conditions on crime scene which has to be maintained in order to preserve the quality of biological sample [4].

In order to release a critical information regarding suspect it is very important for the forensic scientist to distinguish between the different kinds of fibres. Most commonly clothes fibres are encountered on the crime scene are natural or synthetic in nature.

Though blood sample procured from crime scene may be scanty in amount, these days a variety of technologies and tools are available in Forensic Sciences that can be used to extract and greatly amplify the amount of the DNA sample in the blood. One such amplification technique is the PCR. In the DNA isolation technique, stored blood samples are firstly carefully placed in appropriately sized centrifuge tubes. In the next step, the cells present in the blood are lysed to help the proteins and other cellular components break out from the membrane. The cells are treated with a suitable lysis buffer (ammonium chloride being the main ingredient) and a suitable detergent such as TritonX. The tubes are then incubated at room temperature for a few minutes, centrifuged at about 10,000 rpm and the supernatant is discarded. The supernatant is then collected, and is again subjected to RBC lysis followed by incubation and centrifugation. The white pellet so obtained after this process is the white blood cells that contain the DNA. In the next step, the white blood cells are lysed using a WBC lysis buffer (Tris-Cl and EDTA being the key ingredient) and SDS (or Sodium Dodecyl Sulphate). The tubes are then incubated, centrifuged and the supernatant is collected. The DNA present in the supernatant is precipitated out using alcohol and stored in TE (or Tris-EDTA) buffer. Once the DNA is isolated, it can be quantified or visualised using agarose gel electrophoresis [12].

Review of literature

In the mid 1980s analysis of DNA within criminal justice system was established. With this revolution, a minute blood or body fluid can be analysed in a criminal investigation. In field of forensic science the development the DNA analysis increases the accuracy, hence become an invaluable tool for exonerating individuals who have been wrongfully convicted.

Mado Vandewoestyne (2013) had worked to evaluate the assay used to visualize blood on forensic evidences. During his work he had taken blood sample from healthy donor and collected it in an EDTA solution. Firstly he has performed his experiment on undiluted blood sample to visualize blood stain on dark fabric. He performed Km test in which he prepared a phenolphthalein reagent by mixing phenolphthalein, sodium hydroxide and zinc powder in distilled water. They have boiled the solution and added ethanol & distilled water to it. After rubbing the filter paper to blood stain sample they added hydrogen peroxide to the filter paper, followed by phenolphthalein reagent. They had

seen that a pink colour immediately appeared on filter paper indicating the presence of blood [14].

Jakovich (2007) performed a lumiscene test to visualize blood stain on biological evidences. He had prepared a lumiscene reagent by adding lumiscene in milliQ water. He had used activation tablets and stirred the solution at room temperature. When he added the lumiscene reagent to the blood stain sample he had seen a visible light range of 545nm from blood sample which can be seen by naked eyes [16].

Bharadwaj and his co-workers (2011) studied on blood stain identification and screening of type of blood, the methods used conventionally in forensic labs in criminal investigation are not appropriate. In order to identify the real suspect in a criminal case it is important to analyse the DNA. He had used phenolphthalein test to identify blood stain. In his study, with the help of cotton swab he had taken blood from evidence sample and then added it into ethanol + phenolphthalein solution. They concluded that adding phenolphthalein reagent to blood stain sample after a peroxide drop, a bright pink colour was appeared which is shows the presence of blood.

Khusbu Khatiyar (2009) has identified the blood stain in different environmental condition. In her study, she has prepared blood stain sample on different type of fabric. Every fabric has different absorption rate. She has performed KM test to identify the presence of blood stain in fabric sample at different environmental condition. In her study, she has put the sample at different pH [acidic (HCL) and basic (NaOH)], at different temperature [deep freeze, freeze, heat oven & sunlight and at room temperature. She has concluded that in control environment all the sample show positive test. In sunlight, only cotton sample showed positive test. When test was performed on sample kept at deep freeze and freeze condition, they show positive test but take some time to show pink colour. When test was performed on sample kept at different pH samples shows light pink coloration after 2 seconds. Lastly it was conclude that different texture of fabric effect the identification of blood stain [3].

Cheesemann (1995) performed his study on visualization of blood on crime scene stated that blood stain emitted light of 500-590 nm on exposure to fluorescence reagent which can be seen by forensic goggles. He had performed an experiment in which he prepared a fluorescence stock solution by adding sodium hydroxide, fluorescence powder and zinc in distilled water. In the other test tube he had prepared an oxidant solution of hydrogen peroxide in distilled water. In order to make fluorescence working solution he added a ml of prepared stock to oxidant solution. When they sprayed working solution on the sample fabric they have seen a light emerging from blood stain with help of forensic goggles [15].

Vandewoestyne (2013) experiment suggested that it is very important to extract DNA from the evidence found on the crime scene. In his study he has cut the part of fabric containing blood stain and incubated it at room temperature. After incubation fabrics were removed and he centrifuged & re-suspended the pellet on chelex solution. He again incubated & centrifuged the sample and collected sample for PCR.

Andelinovic (1996) stated that the state of the blood sample is likely to interfere with the analysis process and it has been observed in the past that in many criminal investigations,

dried blood stains collected from different fabrics which were procured from the crime scene, had affected the outcome of the examination. Certain changes in the environmental pattern such as temperature change, presence or absence of moisture, contamination etc as well as physical characteristics such as the texture or material of the fabric from where blood has been isolated can grossly interfere in the analysis process and often produce false positives and thus significantly hamper the progress of the investigation process. Thus it is important that we take these factors into consideration while performing blood stain analysis [9].

DH Bing (2000) stated that there are various activities involve in the analysis of DNA like evidence examination, body fluid identification and DNA extraction etc. Extraction or isolation of DNA in its pure form is the ultimate goal of forensic scientist. Traditionally phenol and chloroform are used to isolate DNA from the sample. But now a day's DNA extraction kits are available in the market. Blood, body fluid, semen, hair etc are the forensic evidences found in the crime scene that are not uniform in nature hence it create multiple challenges. It was often seen that body fluid are present as dried strain on different substances which mixed with PCR inhibitors or cause degradation of DNA. The goal of DNA isolation process is to extract more amount of DNA from limited sample source in its pure and stable form which is free from PCR inhibitors in order to detect the identity of suspect etc [12].

Walsh and Hummels (1997) suggested that once DNA has been successfully isolated, a technique called DNA fingerprinting can be employed to ascertain the identity of the individual whose DNA has been isolated from the crime scene. To do this, DNA samples are isolated from the blood of suspected individuals. The DNA from the crime scene as well as the DNA from the suspects are then digested with one or more common restriction enzymes. Restriction enzymes are extremely specific in nature and will only cut at specific positions within the DNA. Once digested, the DNA samples from the suspects are resolved alongside the DNA from the crime scene in an agarose gel. Obviously no two individual's DNA will be the same. Therefore the restriction digestion of the DNA samples will also yield different DNA stretches and therefore different band patterns. That banding pattern which matches with the pattern of the DNA from the crime scene is the culprit. In this way, identity of individuals can be confirmed using DNA analysis. Alternatively, identity of the suspect may also be determined using PCR and the same principle is applicable for this technique as well.

Conclusion

In forensic sciences, blood samples that have been obtained from a place where a crime has taken place, is considered very imperative in analysis. Blood can be collected in dried or liquid form from the crime scene. Mostly dry stain of blood is found on clothes. Using chemical reagent identification of blood stain is done. Different test used to identify blood stain during a criminal investigation by forensic scientist are:-

1. Kastel-Mayer test
2. Benzidine test
3. Lumiscein test
4. Fluorescence test

The Kastle-Meyer test is ideally performed in laboratory to identify blood stain. When hydrogen peroxide was dropped on the blood sample, due to the catalyze activity performed by the heme centre of blood the hydrogen peroxide was reduced to water molecule and release bubbles of oxygen gas. The above reaction causes depletion of electron in heme centre of haemoglobin. When a drop of phenolphthalin was added on the above sample, it donates electron to haemoglobin by reducing itself to phenolphthalein and produce intense pink colour. In this reaction phenolphthalein was not directly involved [5]. In lumiscein and fluorescence test, examiner is able to identify the stain of blood on the criminal biological sample using forensic goggle or naked eyes.

There are many methods which are used to extract DNA but the choice of extraction technique depends upon the type of criminal case like criminal case, sexual assault etc. In criminal investigation it is very important to remove PCR inhibitors from the sample as it can interfere in efficient PCR DNA extraction process.

There are some common steps used in every DNA isolation process:-

1. Lysis of cell
2. Isolation of DNA from cell material
3. Collection and concentration of DNA

After the identification of blood stain, sample of blood is treated with some detergents that disrupt the cell membrane of a cell sample. There are other methods also like physical disruption of cell through grinding etc, but they are not used widely during isolation process. Most commonly chemical methods are used to disrupt cell membrane like ionic, non-ionic and chaotropic agents are widely used for cell lysis. Lastly by ethanol all the content of cell spilled. Firstly lysis of RBC is performed with the help of RBC lysis buffer, then using WBC lysis buffer white blood cells are lysis and DNA is isolated. In small laboratory extracted sample DNA was loaded in agarose gel and run for 45 minutes. After electrophoresis the gel is visualized under UV rays where clear bands of DNA can seen [12].

There are different techniques like agarose gel electrophoresis, extraction using PCR, magnetic separation techniques etc. Hence extraction of DNA from blood sample became easy in laboratories in present world. If the sample found on the crime scene is scanty in amount, then through PCR it can be amplified. In Agarose gel electrophoresis, sample DNA is run under the influence of 80-100v electric field in an agarose gel for 45 minutes. Separation of DNA, occur on the basis of molecular weight. DNA is negatively charge molecule so it moves towards negative electrode. Before gel electrophoresis against a standard (found DNA on crime scene) the DNA samples (suspects) are digested with restriction enzyme so clear band of DNA can visualize under UV rays. In this way blood identification and DNA analysis is perform in criminal investigation by forensic experts. Blood sample procured from crime scene may be scanty in amount, these days a variety of technologies and tools are available in Forensic Sciences that can be used to extract and greatly amplify the amount of the DNA sample in the blood. One such amplification technique is the PCR. In forensic sciences, blood samples that have been obtained from a place where a crime has taken place, is considered very imperative in analysis and criminal investigation.

Blood essentially contains all the information (in the form of DNA as well as certain other chemical compounds) that is needed to narrow down possible suspects and also to determine the identity of the true criminal. There are different ways a forensic expert can interpret the analysis results of blood stains. From the blood stain DNA is isolated and analysis. Techniques like PCR enables the scientist to make copies of sample find on the crime scene. DNA samples are isolated from the blood of suspected individuals. The DNA from the crime scene as well as the DNA from the suspects are then digested with one or more common restriction enzymes. Restriction enzymes are extremely specific in nature and will only cut at specific positions within the DNA. Once digested, the DNA samples from the suspects are resolved alongside the DNA from the crime scene in an agarose gel. Obviously no two individual's DNA will be the same. Therefore the restriction digestion of the DNA samples will also yield different DNA stretches and therefore different band patterns. That banding pattern which matches with the pattern of the DNA from the crime scene is the culprit. In this way, identity of individuals can be confirmed using DNA analysis. Beside this data bases of DNA profile of culprits are extensively coordinated nowadays. It is likely that numbers of suspects will increase in future so the present result of blood stain identification and DNA analysis will ultimately help the future investigator in various criminal investigation^[13].

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