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ORIGINAL ARTICLE

Impact of Degradation of Blood Samples on RNA, DNA and HB: A Review

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ABSTRACT

CONTEXT: Collection of biological evidence from various crime scenes for identification purpose is an important task to achieve. Where different biological evidence serve as an important source of information, whole blood samples and bloodstains act as prime source of identification. Blood samples are mostly found to be exposed to various environmental conditions, which results in the degradation of samples. It is quite inaccessible for forensic experts to re-evaluate the evidences and get the same outcomes as earlier because of storage conditions of the biological samples. Hence, the stability of nucleic acids and hemoglobin is very crucial. This study was conducted to present a review on the available facts of different storage conditions and temperature on the stability of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and hemoglobin (Hb). The study also provides an outlook for ideal storage temperature and conditions for blood samples and appropriate preservation of same immediately after the collection from the crime scene.

KEYWORDS | RNA, DNA, haemoglobin, extraction yield, degradation, storage, forensic analysis

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INTRODUCTION

Various biological evidence redeemed from the crime scene are useful evidences for the purpose of identification and individualization. It is also useful in creating a linkage between crime scene, suspect and victim. Over the last few decades, among all the biological evidences, blood appears to be the prime source for providing abundant source of identification of individuals. Moreover, before the process of collection, the samples are exposed to varied environmental conditions, and whenever the re-evaluation of results is required, the storage conditions have been found to have affected forensic analysis. The purpose of this study is to present a review on the available facts of different conditions and temperature on stability of RNA, DNA and Hb.

Properties of Blood

A circulating fluid that fulfills the nutrition and oxygen requirement of the body is blood. It bears various physical, chemical and biological properties that is to be kept in mind while examining whole blood and bloodstain evidence for forensic examination. Blood falls under the category of liquid connective tissue, which comprises of various blood corpuscles and plasma. Around 7% of total body weight comes from blood in an average human being.[1] Three major types of blood cells and cell fragments are white blood cells (WBCs), red blood cells (RBCs) and platelets. Deoxyribonucleic acid extraction is not possible from red blood cells as nucleus is absent, yet the major element of red blood cell is a protein called hemoglobin which is responsible for oxygen transportation and bears iron.

Blood as Evidence

Blood plays a vital role in identification of a person. It is quite important to ascertain the basic properties and characteristics of blood retrieved from the crime scene. Numerous preliminary examinations are conducted to

endorse if the red droplet encountered at the crime scene is actually blood or not. Kastle-Meyer phenolphthalein test or benzidine test is used to detect and confirm that the sample is blood. Precipitin test is conducted to confirm that the blood of human origin.[1,2] Following this, the individualization of blood is to be done. Having various techniques in hand for RNA and DNA analysis it is quite evident to determine to whom the blood at crime scene belongs. Results of deoxyribonucleic acid analysis authenticate that the blood belongs to the accused or to the victim. Tissue type of body fluid and determination of cell could be identified through the RNA analysis.[3]

Sample Collection

The biological samples need to be carefully collected and stored in a way so that all useful information can be acquired from the analysis. Evidence that can be collected for the purpose of DNA isolation and analysis are of biological nature as mentioned below:

- *Blood and Bloodstains*
- *Semen and Seminal stains*
- *Saliva*
- *Urine*
- *Hair*
- *Bones and teeth*
- *Cells and tissues*

The transfer of biological samples either through direct contact or through the secondary transfer will always be present on the target surface. The collection process must be followed based on the physical properties of the evidence, the liquid state evidence will be present as the absorbent and the solid state evidence will adhere at the surface. For the purpose of acquiring the sufficient amount of DNA from the biological evidences, each sample must be collected and should be stored in dry and cold environment until it reaches the forensic laboratories.

Blood and Bloodstains

Blood and bloodstains appear to be the prime source for providing the high degree of information from hemoglobin, RNA and DNA analysis. The collection process of blood samples from the crime scene is dependent on the surface from where it is to be collected.[4,5]

- *Collection from a Person* This is of great importance to collect a sample as control or for reference from either suspects or victims. While collecting the sample, every tube bearing the sample must be labeled with time, person's name, date, collector's name, case number, location of collection and exhibit number. Blood should be collected by a qualified medical person and in vacutainers with a suitable anticoagulant. Samples must be refrigerated and forwarded to the laboratory. Rahilla *et al.,* (2017) has collected the samples using 5ml disposable syringe and transferred into 6ml K2 EDTA vacutainers and stored in various temperatures in order to assess the influence of storage methods of whole blood samples on integrity of DNA.[6] Di Pietro *et al.,* in 2011 collected the whole blood samples in 1.5 ml of eppendorf tubes of heparin.[7] Schröder and Steimer (2018) also one of the researcher who conducted the study on long term impact of storing samples on DNA methylation and extraction yield by collecting EDTA blood from different individuals and stored at varied temperatures for different durations.[8]
- *Blood in liquid state* Liquid blood must be collected using clean syringe or using spatula in a clean test tube. With proper labeling as mentioned above should be refrigerated and submitted to the laboratory within limited time. Permenter *et al.,* (2015) collected samples and stored using two different preservatives viz. heparin and EDTA at different temperatures and the impact on DNA degradation was assessed for this study.[9]
- *Wet Bloodstains* Small articles like cloth, weapons etc. containing wet bloodstains must be first air dried and then collected and transported, where the large articles like cupboards bears the wet bloodstains, there the wet bloodstains must be transferred to the clean cotton cloth and kept to air dry.

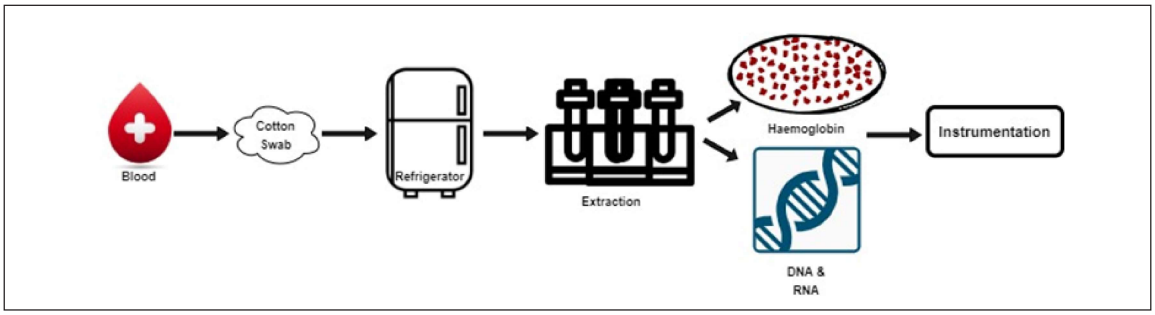

Figure 1: Schematic representation of blood collection, storage, extraction and instrumental analysis

After packaging with a paper container all the objects should be labeled and transported to the forensic lab. Kaur *et al.,* (2020) utilized the blood samples that were soaked using cotton gauze from different crime scenes for the purpose of quantification of DNA and Hb using UV-Visible spectroscopy.[10]

Dry Stains: Bloodstains on small articles like weapons, cloth etc. must be collected independently, packed in paper and transferred to forensic lab. Whereas the articles that are not possible to be collected like from wall, floor or any other non-movable object the samples scrapings should be collected in a paper or tape lift method. If the scrapings lifting is not possible than the dried bloodstains should be collected using saline cotton swab. Then the cotton swab should be air dried and packed in paper envelopes. For the purpose of collection of control an unstained scraping should also be collected from the article.[11,12,13] Stored bloodstains at varied temperatures by absorbing the blood on cotton gauze to analyze the effects of different storage conditions on forensic examinations of blood. Zupanic *et al.,* (2019) stored the samples in varied forms where one of the form used were FTA cards for the blood samples collected at autopsy.[14]

Storage Conditions and Temperature

For the purpose of transportation and storage of evidence which is important for forensic analysis, samples must be secured in paper envelops with proper sealing and labeling. Samples that may bear nucleic acid must never be placed that have warmer conditions or in contact to direct sunlight. All the wet bloodstains collected from the crime scene should be air dried and heat should not be artificially provided to facilitate the dry state of evidence. All the evidence should be kept separated to one another so as to decrease the possibilities of contamination. Various studies were conducted where the samples were stored at specific temperatures like room temperature, 4°C, -20° C and -80°C.[6,7,9,11,12,13,14,15,19] Another study conducted by Howlett *et al.,*. (2013) storage temperature for the blood samples were kept at -20°C, +37°C, +50° C and room temperature. He also used bio stabilizer viz. DNAstable™ for storing the blood samples at varied temperature.[18] Kaur *et al.,* (2020) has considered the dry bloodstains and stored the samples in the form of cotton gauze.[10] Several storage conditions could also be considered for the purpose of analyzing the effect of storage temperature and conditions on degradation of DNA, RNA and forensic analysis of hemoglobin. Bulla *et al.,* (2016) classified the samples in three major categories on the basis of EDTA coupled with DNAgard Blood solution.[15] Sirker *et al.,* considered the dry and humid conditions for the storage of temperature.[3]

Extraction

DNA Extraction

Deoxyribonucleic acid extraction methods play a major role in the quantity, quality, purity and integrity of the isolated DNA from varied biological samples. The selection of the method to be used for the purpose of DNA extraction is based on various factors like, type of evidence retrieved, reduction of contamination risk, cost-effectiveness, simplicity and throughput potential. A study has been conducted where the comparison of three in general used extraction methods (phenol-chloroform, proteinase K and silica based extraction) were used.[7] Where the efficiency of Phenol-chloroform was indicated through various findings. Various other studies were conducted for the same where authors also used commercial kits.[3,15,16]

Cell Disruption In various protocols of DNA extraction different process could be followed for the disruption purpose. It could be achieved by using boiling, alkali treatment, enzymatic digestion and mechanical disruption based on the sample through which the DNA needs to be extracted. Cell disruption plays a major role in the good yielding of extracted DNA, as the decalcification process is required to remove the calcium ions from the sample matrix. Most commonly used decalcifying agent is EDTA which is generally used for the bones and teeth samples, where the calcium ions could interrupt in the process of extraction.[29]

Cell Lysis: Cell lysis is required in order to release the DNA from the membranes, which can be conducted using SDS, sarkosyl and guanidinium salts. Where these substances facilitates to destruct the membranes, denature and dissociate the proteins from the strand.[29]

Removal of protein and Cytoplasmic Contents: After the process of cell lysis there is the need to remove the protein and other cytoplasmic contents from the sample matrix, for this purpose proteins and lipids are to be removed by extraction rounds with organic solvents and cytoplasmic contents could be removed by the reversible binding of DNA with any solid substance. Al-Griw *et al.,* (2017) utilized the Chelex - 100 method for the purpose of genomic DNA extraction.[17,29]

Storage of DNA Solutions: Purified DNA sample is mostly stored in TE buffer (pH 8.0), where EDTA is used as the additive as chelating agent. Isolated DNA could be stored at 4°C or at -20°C for short duration and at -80°C for long durations. Where the cycles of freezing or thawing should be avoided to prevent the breaks of single and double stranded DNA. Udtha *et al.,* in 2014 stored the blood samples at room temperature by adding DNAgard blood, which shows notable extraction yield at room temperature.[19] Howlett *et al.,* evaluated the results utilizing the DNAstable™ medium for storing the isolated DNA samples at room temperature with an approach of low budget and effective storage method for extracted DNA.[18,29]

RNA Extraction

RNA has come up as a favorable tool in recent years for identification of body fluids retrieved from crime scene in forensics. Examination for stability of various mRNA markers specific for human blood in diversified environmental conditions and contaminants has been conducted.[21,28] Moreover RNA is also useful in determining the age of bloodstain through the rate of degradation.[5] For the purpose of both RNA and DNA extraction from the same processing RNA-DNA co-extraction is applied[3] where the procedure of extraction is conducted with an approach of extraction of total RNA, as total RNA bears the considerable amount of mRNA. RNA- DNA co-extraction is recognized for providing the simultaneous extraction of good and effective quality of both RNA and DNA for the purpose of forensic identification of body fluids.[29] Various studies have been conducted for extraction of miRNA for the purpose of understanding the impact of storage methods. Because of less molecular weight and length of miRNA it is required to first extract

the total RNA using organic-solvent method and followed by solid-phase extraction method to improve the small amount of RNA.

Hemoglobin Examination

For the purpose of identification of blood various tests viz. Benzidine test, Kastle-Meyer test, luminol test and leucomalachite green test are being conducted for various years.[22]A proteomic approach for identifying various body fluids has been proposed by Kamanna *et al.,* where the biological samples including blood were firstly mixed with ammonium bicarbonate, reduced with 1, 4-Dithiothreitol, than alkylated with iodoacetamide and in the end digested with trypsin at 37°C overnight. All the digested samples were added with uniform volume of saturated α-Cyano-4-hydroxycinnamic acid matrix solution and deposited on MALDI target plate MTP 384. [23]

Instrumentation

Blood and bloodstain evidences encountered at crime scene are of great importance for identification and individualization purpose. Various preliminary and confirmatory tests are being applied that are destructive in nature. [24,25] Different analytical techniques are developed in recent years where to measure the concentration and purity of the sample can be identified using UV-V is spectrophotometer[15,17,7,10,18,19] and RT-PCR.[3,6,13,16,12] Where on the other hand for the purpose of identify the integrity of extracted sample specially for nucleic acids 1% agarose gel electrophoresis[7,9] is conducted. ATR FTR was applied by orphanou et al. for the differentiation between different biological fluids, where albumin and hemoglobin were tested for blood.[26,27]

DISCUSSIONS

Hb, RNA and DNA are three major factors for the forensic identification and individualization. As the chances of encounter the blood as evidence at a crime scene are very high, it is the area of concern that how the blood undergo changes due to various environmental factors, storage temperature and conditions. The recovery of important information with the help of Hb, RNA and DNA is based on the method of collection and storage as well as on the process followed for the extraction of nucleic acids and proteins from the cell. Di Pietro et al.,. when calculated A260/ 280 nm and A260/ 230 ratio, it was found that silicagel spin column and revisited phenol-chloroform gave the purest genomic DNA respectively.[7] Moreover, while conducting gel electrophoresis to assess the integrity of extracted genomic DNA it was observed that revisited and proteinase-k gave more amount of yielded DNA. Although it was noted that the former method of extraction showed lower level of degradation when used. Various studies were conducted where the blood was kept at room temperature by adding bio stabilizers.[18,19,15,17] Hara *et al.,* in year 2016 has stored the blood samples for more than 20 years showing the stability of bloodstains and whole blood samples can be achieved for long term storage by keeping the whole blood samples at -20°C and -80°C whereas bloodstain samples at room temperature, 4°C, -20° C and -80° C, where -20° C and -80° C are suitable for both.[12,13] The samples received at the laboratories are mostly the piece of cotton gauze soaked in blood [10] Kaur et al., in year 2020 proposed a study with appropriate procedure to be followed for collection and preservation of blood samples from the crime scene and its impact on retrieval of information using UV-Vis spectrophotometer technique.

CONCLUSION

Biological evidences are more likely to be found at a crime scene wherein, blood out of all biological evidences acts as more valuable and corroborative evidence. For the purpose of identification and individualization of victim and accused, blood plays a vital role. In India diversified temperature and climatic conditions at crime scene may lead to degradation of the blood samples before the laboratory analysis. Also whenever the re-analysis of the sample is to be done, it is impossible to provide the same

findings as earlier due to the impact of storage temperature and conditions of the sample even in laboratories. To overcome this factor there is a need to identify the actual impact of different collection, storage conditions and temperature on the degradation of three major components of blood, viz. hemoglobin for the purpose of identifying the presence of human origin blood, RNA to analyze the age of bloodstain as well as the type of body fluid and DNA for the purpose of individualization.

Various studies has been reviewed and it can be concluded that the whole blood samples and bloodstains gave best results when stored at -20°C and -80°C. Although the temperature to be maintained is not possible always, for this purpose the blood soaked cotton gauze after air drying can also be used. The instrument and extraction methods, used in mostly cases is UV-VIS spectrophotometer, other techniques like Raman spectrophotometer and FTIR can also be applied for low yielding samples. The most common extraction method being used currently is phenol-chloroform method, which is quite time consuming and various expensive commercial kits are also available for the extraction from degraded samples.

On the basis of various studies it has been observed that there is no specific study available about the impact of various climatic conditions on the rate of degradation of blood evidences.

There is a need to identify an efficient extraction method for extracting the related information from the degraded blood evidences and to formulate the method which is comparatively less time consuming as well as less expensive than the methods being utilized presently. IJFMP

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