

**Review Article**

**SAMPLE-A RAW DATA FOR FUTURE INFORMATION OF LIFE**

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**ABSTRACT**

Gene amplification requires a biological sample which is collected prior to the demands. The sample for such analysis plays a vital role as they serve as a resource for the core material-Deoxyribonucleic acid. A sample for gene amplification or any analysis would be collected prior to the demands and type of analysis. Human blood has been an essential resource of DNA from the commencement of DNA extraction in the 19th century. After then several protocols developed according to the requirement of both samples of different forms and their retrieval as various forms and methods. The sample has various characteristics and prerequisites when collected for gene analysis. Some important characteristics of sample collection methods are significant, which are not widely taken. This work analyses various general sample collection protocols and highlights some of the major characteristics and prerequisites for the sample. This work initiates and delivers to attain the core of genomics by bringing out the basic raw materials importance and consequences on amplification which is highly sensitive.

**Keywords:** Deoxyribonucleic acid, Extraction, Gene amplification, Genomics, Sample, Prerequisites, Consequences

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**INTRODUCTION**

The Human genome analysis requires the biological molecule that act as a core material called DNA. Human blood is widely used as a sample for genome extraction using different methods, which has been evolving since the 1870s an initiation to extract DNA from blood components and discovery of DNA from that experiment where Friedrich Miescher and Seyler collected the white blood cells using bandages, later Meselson and Stahl extracted bacterial DNA by laboratory protocol. Though the response rate for sample collection is comparatively low for PCR analysis blood is a suitable sample [1, 30-35]. DNA is recovered from blood in different forms such as peripheral blood mononuclear cells, transformed cell lines of white blood cells, whole blood sample, bloodstains in FTA paper or cloth, Clotted blood, Compacted blood, Buffy coat layer, plasma, Autologous conditioned plasma, maternal plasma, menstrual blood stains, long term storage and preserved blood samples, amniotic fluid, umbilical cord blood, blood spots etc. The efficiency of analysis and result completely relies on samples and the DNA retrieved from them. Collection of samples for genome analysis is a highly sensitive and ideal step as an appropriate methodology can reduce the risk of fluctuations in the results. Therefore, adopting a method or developing a strategy for different forms of sample for different demands is not ample and stable. Different protocols are developed and evolved to collect the different forms of blood for DNA extraction and analysis. These protocols are developed on the basis of the yield, quality, storage for further analysis of DNA. Also, the protocols for sample collection may vary according to the extraction methods for DNA isolation. Therefore, collection of sample plays a major role in DNA analysis. The samples must be maintained in an aseptic environment so that the variance in the result and its misinterpretation can be prevented.

This sample collection is highly based on certain characteristics like 1) Type of blood collection, for instance, intravenous, pricking on fingertips and earlobes, peripheral blood collection, non-invasive blood collection 2) Collection materials such as vacutainer tubes(plastic or glass), Filter papers, cloth, fabrics, etc. 3)Temperature both during and after collection, 4) skilled phlebotomist and lab technician for handling the samples, 5) transportation facilities if the subjects-person and lab is distantly placed. [36-38] though the results of several sequencing of an organism's genome seem to be easier comparatively on informatics

technically every strategy for sample collection and preparation is ideal importance as meager care can cause fluctuations in results. This work performs a general analysis of different protocols or methods that are carried out and several demands on the collection of different forms of a blood sample by reviewing various strategies followed in genome analysis so far.

**Method**

This review analysis was done by studying different methodologies that were carried out in different gene amplification studies. From these studies, the significance of sample collection processes was extracted. The sample collection, which is a pre-analytical step plays a major role that can be characterized into major requirements for a sample. Thus, various prerequisite characteristics are categorized from different strategies and studies for blood sample collection.

**Major characteristics and studies for genetic analysis**

The extraction of DNA from blood is a common method carried out for genome analysis as blood acts as an ample source for DNA isolation. There are many forms of sources that can be used for DNA analysis such as whole blood, clotted blood, compacted blood, dried blood spots, and stains, etc. The sample plays a crucial role in DNA analysis as it is a fundamental resource for future studies. In general, biological studies outcomes rely on the sample's quality and stability which is considered as primary importance. Therefore, a sample based on their quality, quantity, stability in different conditions is major parameters considered for analysis of DNA. Also, the characteristics required for 1)Subject or the person who gives a sample, 2) Material or tools for collection,3) Skilled technician before collection such as Phlebotomist, lab technicians, etc, 4) Transportation and lab facilities, 5) Processing of samples, 6) Storage of sample.

Several studies show that these properties are very influencing in the analysis, such as.

**Subject**

The subject's acceptance for an invasive method that is drawing blood intravenously or non-invasive collection using filter paper, fabric, pricking, etc varies according to the type, for instance, adult, old age, children neonates, etc. Intravenous collection and pricking are done for whole blood samples according to the amount required

maximally for adults. Though the blood collection using needles and lancets are painful due to venipuncture and tissue which maximally causes discomfort for subjects, and also large quantity is acquired for the extraction [2]. The blood collection using an automatic lancing device is used in several studies where the discomfort is reduced certainly. Some studies show even the physical and mental wellness of subject is important during sample collection [3]. A large amount of sample is required to sequence the whole genome thus limiting the higher amount of blood collection which is not accepted widely for subjects especially not for neonates. Therefore, pricking on heel or fingertips and acquire a small amount of sample to sequence the whole genome [4]. The non-invasive collection includes painless retrieval using materials such as filter paper, fabrics, which is done by small pricking or drawing, stains deposited on such materials especially dried blood, blood spots, clotted blood are non-invasively collected which is of small quantity but the efficient amount of DNA is obtained. This type of collection makes it comfortable for all grades of subjects and provides easier storage.

#### Materials for collection

Materials or tools for collection of blood sample involves vacutainer tubes-Glass; plastic, filter paper, fabrics, cotton swabs, and clothes. The type of material used for collection plays the main role in providing the quality of sample for analysis; extraction methods; and storage processes.

#### Materials for invasive collection

The whole blood is collected through vacutainer tubes treating them in anticoagulant such as heparin that can be treated which were later with Sodium Iodide-Nal to extract DNA of high molecular weight [5]. The blood sample collection with the help of vacutainer tubes (commercially available BD vacutainer collection tubes) [36]. The whole blood is collected from a vein using needles provided with the collection kit or ordinary needle of the required amount, for example, 2 ml into the sterile vacutainer. There are different vacutinners containing anticoagulants-EDTA; Heparin; Citrate of the required amount to obtain desired DNA quantity and quality. Some study also shows the presence of anticoagulant in the vacutainer affects the resultant DNA [6]. Lippi *et al.* study shows that heparin and EDTA are stronger anticoagulants but comparatively EDTA takes longer time to coagulate. Collection tubes especially the evacuated tube with the needle which prevents the sample exposure to the outer environment and its related changes such as contamination or change in extracted quantity and quality [3]. Hansen *et al.* found the intravenous blood collection in sodium citrate anticoagulant vacutainer tube and stored at 4°C which was compared with other samples like buccal cells, saliva, etc but blood gives 100% purity and high concentration of DNA [1]. Water's *et al.* study on Blood collection kits in P100 tubes and K2EDTA with mechanical separator for compacted blood collection and after collection the sample was cryo-preserved for further DNA extraction. Blood from EDTA tubes is also cryo-preserved as such for DNA extraction. The study shows that both yield and also degradation is higher in EDTA treated sample whereas concentration is higher in P100 treated sample and purity is uniform [7]. Whereas to avoid such degradation due to anticoagulants Lahiri *et al.* used EDTA tubes for blood and added a buffer in order to prevent degradation of DNA sample [8]. The study by Bowen *et al.* shows even the type of tubes has an effect on blood samples for analysis that depicts changes in results due to collection material like glass and plastic tubes [9]. The blood collection for several laboratory purposes has made demanding of tools, therefore several adaptations and evolution to reduce the existing demands in collection tools like glass to plastic for easy portability and disposal, etc [10]. These tubes contain separating gels of the varied amount according to the process needs to be carried out. The determination of specific collection tubes relies on the experimental part and environment in which it is performed. The same author has performed a study on the validation of best tubes for blood sample collection [9]. Stargel *et al.* study proves that the container stoppers that contain drug or chemical bring analytical errors in results from plasma samples of blood [11]. Whole blood sample in vacutainer also depends on temperature, transportation and storage conditions.

The principle behind the collection of biological samples for genome analysis is to collect, process and store for future analysis. But these biological samples require processing, analyse the quality, storage feasibility and also the cost-effectiveness [12]. Genetic profiling using circulating and genomic DNA from preserved whole blood sample which is collected using EDTA vacutinners. A comparative study also determining by storage of sample more than 24 h in EDTA, isolated plasma within 1 hour and at different intervals which showed a significant increase in the amount of circulating free DNA and genomic DNA yield [13]. The yield of mononuclear cells from blood estimates between sample collection from EDTA tubes and undiluted tubes, in which the former is higher than the later. This highly depends on the collection and washing processes of the samples carried through. Where the isolation of lymphocytes is done through NaCl method (dextran sedimentation) [14].

#### Materials for non-invasive collection

The DNA from DBS (dried blood spot), stains from cloth, and clotted blood due to smaller quantity the analysis is highly sensitive because important material has to be extracted from a limited sample. Therefore, respective materials such as Whatman filter paper, Guthrie card, cloth, etc are used for blood collection. For instance, dried blood spot is less prone to contaminating factors and reduces the risk of discomfort because it is less invasive than liquid blood [15]. Gaviria *et al.* study shows that transportation becomes easy for these samples, and storage is also feasible [2]. The Blood collection through filter paper in the form of dried blood spots for detection of diseases especially chromosomal aberrations in newborn babies and other metabolic diseases. The filter paper blood collection device assures quality for samples and results and the studies are done non-invasively [16]. Collection of the blood sample from filter paper for genome analysis and also genotyping of pathogens. Though some study like Bereckzy *et al.* shows major limitation for PCR that requires high purity, quantity, stable DNA from the samples which are lacking in the filter paper method [17]. The blood spots and clotted blood requires additional tools for collection using tools like forceps, Blade, the scalpel to scrap but the technicians are exposed to blood and vice versa therefore contamination is most likely to happen and the person is susceptible to contaminated blood. Certain study like Hollegaard *et al.* in which collection of venous blood is done in heparin vacutainer tube and then the fraction is added to Whatman specimen collection paper and stored for 3 y at -20 °C. Later these punches from filter paper (2\*3.2 mm) were taken for DNA extraction [18].

The genome analysis is also carried out from stained or dried blood from a vein and menstrual release using cotton swabs and clothes that are prepared according to the extraction method adopted [19].

#### Skilled technician

The personnel contamination is a major limitation in sample collection for DNA analysis. Therefore, highly skilled phlebotomist and lab technicians who take this process to the next level by handling the specimens safely is important. This ensures better handling of tools, subject to draw blood and process the specimens with proper care and bio-safety [1]. Personnel safety during collection is a part of pre-analytical characteristic, which is significant on the outcome of the analysis.

#### Transportation and lab facilities

Transportation and lab facilities are often limiting factor in sample collection because the time-lapse in porting the specimen to labs may affect the quality of the samples. The site of sample collection and lab must be situated closer in order to avoid the error or prior arrangements should be done, such as sample preservation in cold temperature till processing.

#### Sample processing

Processing of sample involves treating samples with different anticoagulants and centrifuge to separate the required type of sample, for example, peripheral blood mononuclear cells are separated by centrifuging at a particular speed inappropriate time to obtain different layers of samples. Lippi *et al.* found centrifugation of

sample also plays a major role wherein the difference in speed and time changes the quality of the sample that affects the analysis of the genome [3]. Certain studies show the pre-analytical characteristics as 1) Study design, 2) Sample collection, 3) Handling, 4) Pre-processing, 5) Treating with buffers and other chemicals to maintain the sample for further processes [20].

### Storage

The storage of the sample until the analysis is an important and rate-limiting step because upon improper storage conditions samples tend to lose its quality which affects the findings. The sample storage in short terms of immediate analysis or long term in biobanks are important for a polymerase chain reaction, epidemiological studies, sequencing, etc [21]. The protocols for storage of samples such as blood and tissues at different freezing temperatures are done to preserve the DNA and RNA for analysis such as genome, proteome, etc to diagnose and provide precision medicines. This method has evolved over the years to improve the medical field and well-being. This highly relies on the collection tubes and other standard procedures that are followed in biological sample banks; accordingly, the quality and results are obtained [22]. The Blood samples are best when defibrinated that is without platelets, aliquoted and stored at the appropriate condition like liquid nitrogen, persistent tracking of pre-analytical tools and using documents for quality control and assurance is important [23]. Biological sample especially blood for forensic studies in crime cases, archaeological studies, are limited in quality and quality. Thus, the change in physiological characteristics of samples during and after the collection due to lack of care, contamination, temperature, etc can cause fluctuations in the DNA analysis by changes in DNA information due to distortion, degradation, etc. Generally, DNA is damaged due to environmental disturbances like

physical properties, microbes which contain nuclease that acts on DNA. That is maximum samples DNA degraded within a few days due to exposure to sunlight or UV, temperature. Suitable temperature for DNA is observed to be low (+8 °C) and decrease in success rate by quarter % when stored at 27 °C and hydrolytic damage at humid, Personnel care for preventive measures to collect samples, handling, etc. Storage at a cold temperature (-20 °C and -80 °C) is preferred maximally that cease enzymatic reactions, also dry storage methods with FTA paper can be done to store. Lastly, liquid preservatives can be used such as DMSO, EDTA, Tris [24].

Pietro *et al.* study show the storage of samples for the long term that is even decades in cryopreservation condition also yields better DNA for analysis [25]. Dried blood spot samples from newborn babies as a source for DNA extraction and screening for diseases has developed and this method enables storage for many years. These samples are stored in sample repositories and can be used for future sequencing [18]. DNA isolation from whole blood stored at room temperature has the same yield as the one stored in -70 °C. Also, this paper concludes that the addition of MgCl<sub>2</sub> and EDTA to DNA samples helps in reducing the risk of degradation. The rapid change in storage temperature is causing degradation in DNA [26].

DNA from whole blood that is stored in the long term (15-20 y) at -20 °C using organic solvents like the phenol-chloroform method [25]. The serum yields more DNA than plasma that is the circulating DNA called cell-free genomic DNA is high in serum and plasma in some conditions like systemic lupus erythematosus etc. Results of Lee *et al.*, prove that the yield of DNA from serum is quarter folds higher than plasma, especially from clotted blood which had an increasing amount of circulating free DNA [2].

**Table 1: Analysis of the sample and its related components**

Sample	Sub-categories of sample	Sub-sub-categories	Materials for collection	References
Blood	Whole blood	Plasma, Serum and Buffy coat	Vacutainer tubes	[1,3,5-14,36]
	Clotted blood	-	Filter paper, Fabric cloth and also retrieved from vacutainer	[1-19,36]
	Dried blood or stains	-	Filter paper, Fabric cloth and other materials using scalpel, knife and blade	[2,15-19]
	Compacted blood	-	Vacutainer tubes with a mechanical separator	[1]

### DISCUSSION

This review analysis of sample collection is about the importance of blood sample collection, its related components, and major characteristics. As several genome analysis starts from sample retrieval the type of sample collected with tools and processed according to different demands on studies. This analysis has taken certain major characteristics or prerequisites of sample collection. The sample is collected invasively through needles from a vein in vacutainer tubes, lancets, etc [2-5, 8]. Non-invasive collection of blood spots, clotted blood, etc using filter paper, fabric makes it possible to retrieve sample if available at limited volume but also shows better results [1-3, 15]. The transportation, processing, and storage of collected process are potential properties to be taken care of because lack of care in these characteristics result in improper outcomes [1, 9, 20-21]. Despite many analyses and their successful data produced as an outcome, their success rate relies on the way of the collection of samples and their bio-safety practices carried out. The data loss or modified data, errors in data are due to the lack of care on samples which are considered to be simple and easy but their impact affects the analysis as this is highly sensitive process and extraction of the information [27, 28]. The pre-analytical characteristics, such as sample processing need to be monitored initially to avoid experimental errors on resultant data [29]. Despite many protocols for sample collection, choosing a standardized strategy is challenging and also many research works do not explain completely about the importance and protocol of sample collected [30]. Thus, making it difficult to extract the common principle for sample collection, especially blood. Certain demands such as proper standardization of universal strategy for sample collection is not

available, as it not completely clear for a particular sample. The future studies need to be confirmed and concentrate on the sample as it is the raw data.

### CONCLUSION

This review analysis shows the importance and categorizes different pre-requisites of sample collection and also highlights the blood and its related components with materials for collection. It concludes that sample-blood is the ample and core source of genome analysis which is universally used in many experiments and its characteristics according to various demands.

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### AUTHORS CONTRIBUTIONS

The authors' AA, VS and had contributed equally towards the collection of literature and preparation of the manuscript.

### CONFLICTS OF INTERESTS

Authors declare that they have no conflicts of interest.

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