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Procurement, storage, and use of blood in biobanks

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Summary

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Blood is a widely used biospecimen in the field of biobanking, secondary to the ease with which it is collected along with the wide variety of analytes obtained from it for analysis. It carries the potential to further the search for biomarkers in countless diseases; therefore, the standardization and optimization of blood collection procedures is of importance in assuring reproducibility of results. Here, we briefly review procedures for the procurement, storage, and use of blood and its fractions for biobanking purposes. Select commonly used methods for collecting blood using various vacutainer blood collection tubes are described, along with methods for optimal storage of various samples in short- and long-term situations.

Keywords

Blood; Biobanking; Procurement; Storage; Plasma; Serum; Biomarkers; Biospecimen; DNA

1. Introduction

Blood is one of the most commonly used biospecimens in biobanking and plays a major role in the search of new biomarkers and the early detection and treatment of complicated diseases, such as cancers (1, 2). In comparison to other biospecimens, blood is readily obtained, easily handled and perceived useful for testing by the public (2). Its various components also provide a multitude of possible applications such as analyte studies, flow cytometry and culture experiments among others. With such versatility in its uses and such significance in its implications for translational research, it is desirable that blood be optimally collected and stored.

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The proper methods for collection and storage of blood depend almost entirely on the desired fraction and the intended use of the sample (1). Distinct colored tops of the collecting tubes indicate the type of additive present. These additives help isolate the individual fractions of blood needed for testing (1), after the blood undergoes many rounds of centrifugation. Some of the most commonly used blood components are:

1. plasma; liquid part of blood that remains after the suspended cells have been centrifuged; contains clotting factors and has dissolved electrolytes,
2. serum; similar to plasma but without the clotting factors,
3. buffy coat; fraction of blood that mostly contains white blood cells and platelets, and
4. red blood cells (1–3).

The uses and storage requirements of each of the fractions of blood are listed in Table 1.

It is important to recognize the fraction of blood needed for the purpose of the research so that appropriate collecting tubes are used. There are a large number of tubes with different stabilizers and the reader is referred to commercial vendor websites to decide upon the specific one appropriate for your use. These are typically highlighted by a specific color cap. However, if the aim of the research is unclear, collection of blood in a tube containing K^+ EDTA can provide ample DNA which is the most common need currently (Table 2). In particular, for cancer banks, blood DNA is useful as a germline control. It is impractical and costly to collect multiple different blood tubes without a clear goal. In addition, if a specific analyte is of interest for a specific study, it is often important to keep the methodology of procurement, handling, and storage as uniform as possible. For example, transportation of blood tubes on ice instead of at room temperature can be beneficial for some analytes but can be deleterious for others. A detailed record of the collection and storage protocol is potentially helpful to explain discrepancies that might skew an analysis. For simply obtaining DNA for sequencing, the transportation protocol may affect yield but should not affect sequencing analyses. However, measurement of metabolites and other analytes may be vulnerable to protocol variations.

2. Materials

All blood specimens should be considered infectious and therefore personal protective equipment such as disposable gloves and protective goggles should be used during blood collection and handling.

2.1 Collection Materials

1. An evacuated tube system with interchangeable tubes containing additives for individual applications (4):
   a. 2–10 ml tubes containing Na^+ EDTA,
   b. 3–8 ml tubes containing lithium heparin (LH/PST),
   c. 3.5–10 ml tubes containing silica clot accelerator (SST),

d. 6–8.5 ml tubes containing acid citrate dextrose (ACD) etc.

2. Centrifuge with swinging bucket rotor
3. Disposable pipettes (2 ml or less)
4. Cryovials with labels
5. Ice
6. Small ice bucket
7. Dimethyl sulfoxide (DMSO): 20%, diluted with growth medium

2.2 Storage Materials

1. Water and frost resistant labels (−80 °C)
2. Laboratory Information Management System (LIMS) or equivalent data record system
3. Programmable cell freezer
4. Liquid nitrogen
5. Refrigerator (4 °C)
6. Freezer (−80 °C) with monitoring system
7. Specimen boxes or 96 position racks

2.3 Analysis Materials

1. Polypropylene tubes (15 ml)
2. Lysis buffer: 10 mM Tris-HCL, 400 mM NaCl, 2 mM Na^+ EDTA, pH 8.2
3. SDS 10%
4. Proteinase K solution: 1 mg proteinase K in 1% SDS and 2 mM Na^+ EDTA
5. Centrifuge
6. Absolute ethanol
7. TE buffer: 10 mM Tris-HCL, 0.2 mM Na^+ EDTA, pH 7.5
8. Gilson pipette

3. Methods

Blood is often fractionated before being analyzed and stored. Therefore, determine the samples' intended use and follow instructions accordingly to obtain the appropriate fraction/s of blood needed.

3.1 Blood Collection

Whole blood will be collected from patients by a phlebotomist to avoid causing study participants discomfort and/or compromising the quality or quantity of the sample (5).

Collect blood in appropriate collecting tubes with the help of vacutainer collection system. Blood collection tubes should be drawn in a specific order to avoid cross-contamination of additives or in order of priority of assay for which tubes are needed (6). It is critical to cross check patient information before labelling the test tubes. Some blood analytes are stable at certain temperatures while others are labile. Therefore, it is important to follow uniform practices of transporting and storing blood and its fractions, in order to avoid drop in biomolecule yield (1). All samples may be transported at 4 °C (on ice), unless otherwise stated.

3.1.1 Na^+ EDTA collection tubes—These tubes are used for collecting anticoagulated blood and suitable for DNA assays.

1. Invert the tube 8 times to prevent clotting.
2. Centrifuge at 1100–1300 g for 10 minutes at 4 °C (*see* Note 1).
3. Using disposable pipettes, transfer desired aliquots of plasma, buffy coat, and/or red blood cells into labeled cryovials (*see* Note 2 and 3).
4. Tightly cap and place on ice until ready for storage. EDTA samples intended for hematological assays should be kept as fresh blood and need not be transferred into cryovials (7).

3.1.2 Lithium Heparin/PST tubes—These tubes are used for collecting plasma which is mainly used for proteomic studies (*see* Note 4).

1. Invert tube 8 times to prevent clotting.
2. Transfer aliquots of plasma (separated by the gel separator plug) using disposable pipettes into labeled cryovials.
3. Tightly cap and place on ice until ready for storage.

3.1.3 Silica Clot Activator/SST tubes—These tubes are used to separate serum which is used in the analysis of antibodies, nutrients, lipids, and lipoproteins as well as for proteomic studies (*see* Note 4).

1. Invert 5 times and allow blood to clot for 30 minutes.
2. Transfer aliquots of serum (separated by the gel separator plug) using disposable pipettes into labeled cryovials (*see* Note 5).
3. Tightly cap and place on ice until ready for storage (*see* Note 6 for alternative).

3.2 Blood Storage

After collection, the whole blood or its fractions are aliquoted into cryovials for long-term storage. All samples should be properly labeled, and their location recorded in a database for easy retrieval in the future (*see* Note 7). In general, blood processing should be performed, and samples put into storage as soon as possible to avoid the degradation of any biomolecules (1).

1. Cryovials, organized in specimen boxes or racks, should be temporarily stored in a refrigerator held at 4 °C for no more than 24 hours, as delayed processing of tissue is correlated with RNA degradation (1).
2. Plasma or serum should be stored in mechanical freezers at a temperature of −80 °C (5).
3. Lymphocytes or other cellular specimens should be stored in the vapor phase of liquid nitrogen at −150 °C when long term viability is necessary as these cells can only stay viable at room temperature for up to 48 hours (1, 5) (*see* Note 8 and Note 9).
4. Check storage temperatures at least once daily (*see* Note 10).

3.3 Blood Analysis

Once properly stored in a biobank, samples may be thawed for use in many applications, most commonly DNA or RNA extraction and genome sequencing.

3.3.1 DNA extraction—DNA material is obtained from nuclear material of lymphocytes present in the buffy coat.

1. Resuspend the buffy coat with 3 ml of nuclear lysis buffer.
2. Digest the cell lysates with 0.2 ml of 10% sodium dodecyl sulfate (SDS) and 0.5 ml of proteinase K solution overnight at 37 °C.
3. Shake vigorously for 15 seconds with 1 ml of 6 M NaCl in each tube.
4. Centrifuge at 2500 rpm for 15 minutes. Transfer the DNA (in the supernatant) to another polypropylene tube.
5. Add 2 volumes of absolute ethanol and invert the tubes several times until the DNA precipitates.
6. Remove the DNA with a plastic spatula or pipette and transfer to a 1.5 ml microcentrifuge tube containing 100–200 microliters TE buffer, and dissolve for 2 hours at 37 °C.
7. Store at 4 or −20 °C until ready for use (8) (*see* Note 11 and 12).

4. Notes

1. Centrifuge must be balanced when used. For RCF on-line calculator tool visit: http://www.changbioscience.com/cell/rcf.html (3). Centrifuge speeds over 2000 g may cause the tubes to break.
2. Recommended aliquot volume is less than 2 ml for all fractions. For plasma and serum, 100 or 250 μl aliquots are recommended (3). Separating fractions into small aliquots before storage helps to minimize sample exposure to freeze-thaw cycles during future use, which has been shown to have a large negative effect on specimen quality (1, 2).

3. The plasma layer will be on the top after centrifugation. Underneath will be the whitish “buffy coat” layer, which is composed of mononuclear cells and platelets. The final layer will be the red blood cells (3). Be careful not to aspirate liquid from another layer while collecting the desired fraction in the pipette.

4. Both serum or plasma may be used for proteomic analyses, but for studies intended to investigate the broadest array of proteins and peptides, plasma is the better choice, as the process of blood coagulation in serum collection results in the loss of many proteins.

5. If the serum sample is hemolyzed, it cannot be used (9). This will be visually detected by a pink to red tinge.

6. For alternative method to collect serum: Collect blood in the Red Top BD Vacutainer. Allow these tubes to sit upright at room temperature for 30–60 minutes. In this time, the red blood cells will form a clot. Either immediately or after no more than 4 hours in a refrigerator, centrifuge the samples for 20 minutes at 1100–1300 g at room temperature (*see* Note 1). Using a pipette (do not pour), transfer aliquots of the serum layer into labeled cryovials no more than 1 hour after centrifugation (*see* Note 2). The serum layer will be on the top after centrifugation, followed by the clot. Be careful not to aspirate red blood cells while collecting the serum layer. To do so, keep the tip of the pipette above the bottom layer and leave some of the serum behind in the tube. Tightly cap the cryovials and place on ice until ready for storage (9).

7. Labeling and tracking systems may vary; however, the use of barcodes is the most ideal. Barcodes both protect the privacy of the patient (10) and efficiently connect the sample to any relevant clinical information in a separate program or database (7). To withstand long-term storage temperatures, labels must be water and frost resistant (4).

8. Freeze-drying offers an alternative approach to long-term sample storage. In this three-step process, the sample is frozen to convert the water into ice. Then, the ice is converted to vapor. Any remaining water in the sample is then removed by again converting to vapor. To preserve blood by this process, simply put the samples in a freeze-dryer. Samples that are freeze-dried can then be stored at temperatures above 5 °C; long-term storage in freezers is not necessary (11).

9. Vapor phase liquid nitrogen is preferred over liquid phase storage where cross contamination of specimens is a concern (5). Caution must be exercised when handling liquid nitrogen (−196 °C) which can instantly freeze flesh. Therefore, protective face, hand and eyewear must be worn at all times when handling liquid nitrogen (5).

10. To avoid freeze-thaw effects on samples, storage units should be equipped with a temperature-monitoring system and alarm system to track and send alerts of any temperature changes (1, 7). Along with temperature monitoring the alarm system should also be able to detect nitrogen levels (5).

11. There are numerous other methods for DNA extraction. The traditional gold standard method for DNA extraction is the phenol chloroform method. We have mentioned the alternative method because it avoids the use of phenol chloroform, making it quicker, safer, less expensive, and less environmentally hazardous than other methods (8).

12. A potential alternative for blood collection and DNA extraction in biobanking is dried blood spot technology using filter paper cards that are pretreated to retard bacterial growth, inhibit nuclease activity and release DNA during processing (5). This microsampling substitute can be easily obtained with a heel or finger prick, stored at room temperature, easily transported with little biohazard risk and is a much cheaper option than the traditional blood collection and storage method (12).

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Table 1.

Uses, collection characteristics and storage requirements of the different fractions of blood

Biospecimen	Collection Characteristics	Uses	Storage recommendations
Whole blood	Anticoagulant (ACD, heparin, EDTA); protease inhibitor for proteomics	Genomic studies; source of DNA and RNA	Stored at −80 °C if DNA extraction cannot be carried out immediately
Buffy coat	EDTA	Source of DNA and RNA	Store at −150 °C for RNA stability
RBC	EDTA	Hemoglobin studies Membrane proteomic research	Should be aliquoted and kept at −80 °C
Plasma	Lithium heparin/EDTA	Proteomics; Source of DNA; multiple analyte studies	Stored at −80 °C
Serum	No anticoagulant	Improved analysis of antibodies, nutrients, lipids and lipoproteins	Stored at −80 °C

Table 2:

Select different additives and their respective tube tops

Tube top	Additive
	**EDTA:** for collecting anticoagulated blood consisting of plasma, buffy coat and red blood cells.
	**Lithium Heparin (PST):** for collecting plasma.
	**Acid Citrate Dextrose (ACD):** for collecting anticoagulated blood consisting of plasma, buffy coat and red blood cells.
	**Silica Clot Activator (SST):** to separate serum