

Blood Collection, Shipment, Processing, and Storage

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Abstract

As part of the AACR Annual Meeting Methods Workshop "Sample Collection, Processing, and Storage for Large-Scale Studies: Biorepositories to Support Cancer Research," blood specimen collection, processing, storage, and dissemination issues were discussed. Whole blood and blood fractions comprise a major portion of biospecimen collections for population-based studies. Although procedures for collecting, processing, storing, and shipping blood components are generally standardized and well documented, several important factors need to be considered before a new study involving blood collection is initiated. Blood is collected from study participants for a variety of purposes, including as a source of DNA, and for a variety of laboratory analyses that may be done on whole blood or blood fractions, including

serum, plasma, and lymphocytes. Blood or blood fractions may be shipped to laboratories and biorepositories either at ambient temperature, cooled or frozen, depending on the intended analyses. Blood processing may include fractionation, cryopreservation to preserve the viability of lymphocytes, or purification of nucleic acids. The proper storage conditions depend on a variety of factors, including the intended analyses and whether the specimens will be used within a short period or need to be stored for longer periods. As for all laboratory and biorepository procedures, blood collection, shipment, processing, and storage should be conducted under a strict quality assurance program, including standard operating procedures and regular quality control reviews. (Cancer Epidemiol Biomarkers Prev 2006;15(9):1582-4)

Introduction

The 2006 AACR Annual Meeting Methods Workshop "Sample Collection, Processing and Storage for Large Scale Studies: Biorepositories to Support Cancer Research" included a presentation and discussion of blood specimen collection, processing, storage and dissemination issues. These processes are generally well-understood and documented, but there are many logistical and scientific issues that need to be considered before engaging in a new study involving blood collection (1). In order to preserve blood and blood fractions in optimal condition appropriate for the intended laboratory analyses, all collection and processing steps must be conducted under a well-defined quality assurance program. It is also important to consider new or alternative methods and reagents that may offer longer term stability or increased efficiency in the collection and preservation of blood.

Materials and Methods

Collection. Collection of blood specimens should be carried out by trained phlebotomists to avoid causing study participant discomfort or compromising the quality or quantity of the sample. The study participants should receive clear oral and/or written instructions, with information, for example, about fasting and avoidance of medications as necessary for the planned analyses (2). For blood collection, standard

protocols recommended by well-established organizations must be used (3).

An evacuated tube system [e.g., Becton Dickinson (Franklin Lakes, NJ) Vacutainer; <http://www.bd.com>] with interchangeable plastic tubes is commonly used to collect blood. The tubes, some with additives appropriate to a specific application, are differentiated by their color-coded stoppers (4). Blood collection tubes should be drawn in a specific order to avoid cross-contamination of additives (4).

An important early decision in blood collection is whether to collect anticoagulated blood (consisting of plasma, buffy coat, and RBCs) or coagulated blood (consisting of serum and RBC clot; refs. 1, 5, 6). There are several types of anticoagulants, which differ in their mechanism of action and which need to be chosen carefully to avoid problems with certain laboratory applications. Heparin binds to antithrombin III and accelerates the inactivation of thrombin and other clotting factors. EDTA chelates metals, such as calcium and magnesium, which may be beneficial for some blood-based assays but adversely affect others. As an anticoagulant, EDTA is well suited for DNA-based assays but is problematic for cytogenetic analyses (2). Despite anecdotal accounts of problems in PCR assays, studies have generally found that the use of heparin or EDTA produces equivalent results in PCR assays (2, 6). Acid citrate dextrose also chelates calcium. Citrate-stabilized blood results in better quality RNA and DNA than other anticoagulants and yields more lymphocytes for culture (2). However, in liquid form, acid citrate dextrose dilutes plasma, and a dilution factor will have to be considered when calculating assay results. If variable volumes of blood are drawn from study participants, dilution can result in erroneous results if volumes are not carefully recorded.

Other special collection tubes, such as Serum Separator Tubes and Cell Preparation Tubes (Becton Dickinson), allow for more convenient separation of blood fractions. However, the Serum Separator Tubes have been found to affect some

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assays, such as thyroxin and cortisol (7). Special collection tubes with protease inhibitors have been developed, which preserve proteins for proteomics analyses. The analysis of trace metals in blood also requires caution, as trace metals may be present in the evacuated collection tubes. Lot-to-lot variation in the quality of collection tubes is also a potential source of spurious laboratory results.

Results

Stability. The stability of blood with respect to various laboratory analyses may be affected or controlled as follows (1, 2, 5, 6): (a) anticoagulants used in blood collection as described above; (b) stabilizing agents (e.g., EDTA and ascorbate) are necessary to preserve folates and vitamins and should be included in the collection device or added as soon as possible after collection to assure stability of analytes; (c) the time elapsed between blood collection or removal from a storage unit and subsequent processing; (d) the temperatures at which blood specimens are processed and stored may be important depending on the intended analyses; (e) thaw/refreeze cycles should generally be avoided due to the potential for instability of some analytes; (f) enzymatic degradation affects many biochemical markers, RNA and proteins are particularly susceptible to enzymatic degradation and require special procedures to maintain their integrity during collection and processing, and the addition of commercially available RNase inhibitors secures RNA integrity (2); and (g) the PAX DNA Blood Collection System by PreAnalytiX (Franklin Lakes, NJ; <http://www.preanalytix.com/DNA.asp>) allows for the collection, shipping, and short-term storage of blood at room temperature and for subsequent extraction of DNA according to a single-tube protocol.

Safety Considerations. All blood specimens should be considered potentially infectious, the major risks being exposure to hepatitis and HIV. Universal precautions practices, including prior hepatitis vaccination, should be followed during blood collection and handling. All fresh and frozen biospecimens should be considered potentially infectious (1). The Centers for Disease Control and Prevention and NIH have published a biosafety manual (8).

Dry-State Blood Collection. Blood collected on treated paper cards (available, for example, from Whatman, Brentford, Middlesex, United Kingdom; <http://www.whatman.com/>) is suitable for many laboratory applications and, if appropriate for the intended analyses, will result in less expensive storage conditions. Enough DNA can be obtained from a 2-mm punch of a paper card for ~500 single-nucleotide polymorphism genotypes. In addition, there is a large array of analytes that can be measured from dried blood spot biospecimens for biochemical markers, mutations, or polymorphisms (9, 10). The extraction of DNA from blood spot cards can be automated (11).

Shipment

Depending on whether they are known to contain infectious agents and on the intended analyses, blood specimen shipments may be regulated as infectious substances or as diagnostic specimens. To properly classify the specimens to be included in a shipment, consult references provided in the International Society for Biological and Environmental Repositories Best Practices (1) and by the International Air Transport Association (12).

Blood specimens are often exposed to temperature fluctuations during transit. The following are typical temperature

conditions required for the transport of blood and the insulation/refrigerant required to maintain that temperature. The required shipping temperature depends on the intended analyses (1): ambient (+20°C to +30°C), insulated packaging to protect from extreme hot or cold ambient conditions; refrigerated (+2°C to +8°C), gel packs designed for refrigerated temperatures; frozen (−20°C), gel packs designed for frozen temperatures; frozen (−70°C), dry ice pellets or sheets; and frozen (at or below −150°C), liquid nitrogen dry shipper.

Devices are available to monitor temperature trends during shipment either by recording temperatures precisely at certain time intervals or by changing color if a certain temperature is exceeded during shipment.

Processing. Processing of blood biospecimens depends on the laboratory analyses to be done. Fractionation of blood results in the following components: (a) mononuclear leukocytes (peripheral blood mononuclear cells) are the only cell type in blood that can be maintained in a viable state; (b) neutrophils (the most abundant type of granulocytes) are also nucleated and another source of DNA; (c) erythrocytes can be used to study adducts of hemoglobin; (d) plasma is obtained from an anticoagulated blood sample by separating out the cellular components; and (e) serum isolation requires no anticoagulants. To reduce contamination, separate serum from other blood components as soon as possible. Serum allows for improved analyses of antibodies, nutrients, lipids, and lipoproteins (6). Either serum or plasma may be used for proteomic analyses.

If the fractions are not intended for immediate use, they should be aliquoted into small cryovials and stored in mechanical or liquid nitrogen freezers (5).

Cryopreservation is a cost-effective way of preserving viable lymphocytes for subsequent recovery of DNA or for EBV transformation to create lymphoblastoid cell lines as a source of unlimited amounts of DNA (13). Cryopreservation typically involves the use of a cryoprotectant, such as DMSO. Commercial cryoprotectants that are less toxic have been developed (<http://www.biolifesolutions.com/publications.asp>). Whole blood may also be cryopreserved as an efficient and cost-effective approach to centralized processing and storage of viable cells in large-scale epidemiologic studies (14).

Aliquoting is often necessary to preserve multiple samples to avoid thaw/refreeze cycles. The volume and number of aliquots should correspond to the intended analyses to avoid unnecessary long-term storage.

Storage. Depending on the intended laboratory analyses, whole blood and blood fractions may be stored under a variety of conditions. In general, plasma or serum should be stored in mechanical freezers at −80°C and lymphocytes or other cellular specimens should be stored in the vapor phase of liquid nitrogen at −150°C or lower when long-term viability is necessary. Some general storage considerations are as follows (1). (a) Adequate back-up capacity for low temperature units must be maintained. Personnel must be trained in processes and techniques for rapidly transferring material to back up units when necessary. (b) Where liquid nitrogen freezers are used, an adequate supply of liquid nitrogen must be maintained. (c) Vapor phase liquid nitrogen storage is preferred over liquid phase storage, where cross-contamination of specimens may occur. (d) Alarm systems should be in place to monitor the temperature of mechanical freezers or, for liquid nitrogen freezers, the liquid nitrogen level and temperature. (e) The use of liquid nitrogen poses special safety problems. With a liquid temperature of −196°C, flesh freezes almost instantly if it comes in direct contact with the liquid. Both face and eye protections are

required. Oxygen level sensors should always be used when liquid nitrogen freezers are used in a biorepository. (f) Dry ice is frequently used as a refrigerant for shipping and emergency backup for mechanical freezers. (g) A system for maintenance and repair of storage equipment, support systems, and facilities should be in place. (h) All equipment should be validated before use or following repairs that affect the capabilities of the instrument.

Discussion

Although many prior studies have resulted in well-established procedures for the proper collection, processing, and storage of blood and blood fractions, additional work is needed. (a) For example, among future trends in blood collection, smaller volumes will be required as laboratory analyses become more sophisticated and sensitive. For example, for DNA applications, such as genotyping, we have already seen the requirements reduced from microgram to nanogram quantities in the past 3 years. In terms of collection methods, dry-state storage on treated blood spot cards is likely to become more prevalent as a means to conserve space and reduce storage costs. (b) Although some effects of blood collection tube additives have been studied and documented, the advent of new collection tubes with new variations of additives will require additional study to assure that they do not affect certain assays. (c) The stability of blood fractions with respect to a variety of assays has not been well documented. For each assay, it must be determined if the blood or blood fraction sample is stable under the planned storage conditions (i.e., length of time and temperature).

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