Translational Research in Pediatrics II: Blood Collection, Processing, Shipping, and Storage

abstract

Translational research often involves tissue sampling and analysis. Blood is by far the most common tissue collected. Due to the many difficulties encountered with blood procurement from children, it is imperative to maximize the quality and stability of the collected samples to optimize research results. Collected blood can remain whole or be fractionated into serum, plasma, or cell concentrates such as red blood cells, leukocytes, or platelets. Serum and plasma can be used for analyte studies, including proteins, lipids, and small molecules, and as a source of cell-free nucleic acids. Cell concentrates are used in functional studies, flow cytometry, culture experiments, or as a source for cellular nucleic acids. Before initiating studies on blood, a thorough evaluation of practices that may influence analyte and/or cellular integrity is required. Thus, it is imperative that child health researchers working with human blood are aware of how experimental results can be altered by blood sampling methods, times to processing, container tubes, presence or absence of additives, shipping and storage variables, and freeze-thaw cycles. The authors of this review, in an effort to encourage and optimize translational research using blood from pediatric patients, outline best practices for blood collection, processing, shipment, and storage. *Pediatrics* 2013;131:754–766

AUTHORS: Carolina Gillio-Meina, PhD,a,b Gediminas Cepinskas, DVM, PhD,c Erin L. Cecchini, MSc,a,b and Douglas D. Fraser, MD, PhD,a,b,c,d,e,f

“Translational Research Centre, London, Ontario, Canada; bChildren’s Health Research Institute, London, Ontario, Canada; cCentre for Critical Illness Research, dCritical Care Medicine and Pediatrics, eClinical Neurologic Sciences, and fPhysiology and Pharmacology, Western University, London, Ontario, Canada

KEY WORDS

translational research, pediatrics, repository, blood, serum, plasma, DNA, RNA

ABBREVIATIONS

IATA—International Air Transport Association
POCT—point of care testing
RT—room temperature

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Address correspondence to Douglas D. Fraser, MD, PhD, Paediatric Critical Care Medicine, Room C2-843, Children’s Hospital, London Health Sciences Centre, 800 Commissioners Rd East, London, ON, Canada, N6A 5W9. E-mail: douglas.fraser@lhsc.on.ca

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Translational research studies often investigate molecules in human tissues to better understand disease mechanisms or as either a measure of disease outcome or response to therapy. Blood is by far the most common tissue used, but in pediatrics it is often difficult to procure, mandating that best practices are used to yield quality samples for optimal study. Several factors can affect the quality of the blood samples, including the chosen container tubes, anticoagulants, preservatives and additives, processing times, centrifugation settings, shipping methods, storage variables, and freeze/thaw cycles. Poor attention to details or naive practices can negatively influence analyte stability and cellular integrity, potentially distorting the experimental results. Furthermore, each biomarker or cell type has its own inherent properties, which may require protocol modifications for quality sample preservation.

In this review, our second in a series on tissue sampling and bio-banking for child health studies, we present the pertinent issues that arise from blood collection, processing, shipping, and storage. Our goal is to highlight the different approaches and the best practices to maintain blood samples with the highest integrity. Pediatric-specific issues in translational research, such as study ethics, consent, and sampling volumes, were reviewed by us previously.

**BLOOD SAMPLE COLLECTION**

Inaccurate experimental results are often secondary to inappropriate specimen collection and handling, or to interference factors. Laboratory measurements are influenced by hemolysis (high-negative-pressure blood draws or use of tourniquets), lactescence (nonfasting patients), concentration changes (drawing from an infusion line, insufficient filling of vials altering sample/additive ratio), bacterial contamination (production of ammonia or urea), and extravascular interchanges.

Blood draws from heparinized lines can falsely lower ionized calcium.

One of the earliest considerations is deciding whether unprocessed whole blood, plasma, or serum is required (Table 1). Unprocessed whole blood requires no further processing once drawn from the patient and can be used for “point of care” testing (POCT). POCT offer immediate results that not only can be used for rapid patient diagnostics but also to support research studies. Common POCT includes blood gases, electrolytes, hematocrit, glucose, creatinine, hemoglobin A1C, amylase, cardiac markers, coagulation markers, cholesterol, urinalysis, and streptococcal infection. POCT analyzers can provide precise results that correlate with results from the reference laboratory, but their accuracy relies on attention to quality control and operator training.

Unprocessed whole blood can be rapidly separated to yield either serum or plasma. Serum samples undergo the process of coagulation, which consumes all available clotting factors from the sample but can also impose artificial effects on the levels of biomarkers. In contrast, plasma is collected into tubes that contain anticoagulants to prevent the clotting of blood, and thereby retains clotting factors in solution. Centrifugation of unprocessed whole blood for plasma also results in isolation of auffy coat layer, which can be used to produce cell concentrates (ie, leukocytes) for use in a wide array of in vitro studies or as a source of nucleic acids. Although serum and plasma preparations are often used interchangeably, large differences exist between the proteomes of serum and plasma, making them incomparable to one another. Hence, it is critical that either plasma or serum is exclusively chosen for study. Examples of biomarkers altered by blood processing are shown in Table 2.

**BLOOD COLLECTION TUBES**

Evacuated tube systems for blood collection, such as Vacutainer (BD Biosciences, Franklin Lakes, NJ), are available for the isolation of serum, plasma, and buffy coat RNA or DNA. Frequently used blood collection tubes are summarized in Table 3 and should be chosen on the basis of the end-point assay.

**Serum Isolation**

Silicone-coated tubes contain a clot activator and are useful for serum chemistry. Alternatively, serum-separator tubes contain a gel barrier and decrease the incidence of hemolysis, increase sample stability, and facilitate primary tube sampling and storage. Disadvantages of gel barrier tubes include instability under extreme temperatures and barrier-induced changes in serum drug concentrations. The type of serum collection tube that is selected can also have an effect on end-point analysis due to the differences in clotting facilitated by the additives. Serum tubes may add unwanted polymeric components to the serum, leading to inaccurate results.

**Plasma and Buffy Coat Isolation**

Citrate is a common anticoagulant used in plasma collection tubes, resulting in quality DNA and RNA isolation and high yields of lymphocytes that can be isolated for cell culture. Platelets are stable in citrate, releasing fewer small peptides into the plasma relative to other anticoagulants. Heparin is an alternative anticoagulant used for plasma separation but may alter T-cell proliferation and antithrombin III activity, and protein interactions.
Plasma isolated from tubes containing EDTA as an anticoagulant exhibits changes in protein profiles over time compared with citrate.23

**Blood Cell Concentrates**

After density gradient centrifugation of whole blood, red blood cells can be isolated from the bottom fraction of the tube, and the buffy coat contains leukocytes and platelets. Further isolation of leukocytes and platelets can be accomplished with density gradient centrifugation and/or antibody-coated beads.24

Isolated leukocytes provide a means to assess the inflammatory response to disease. The type of anticoagulant, sample collection, and handling and processing techniques, however, can greatly affect the yield, quality, and morphology of isolated leukocytes and platelets. Further isolation of leukocytes and platelets can be accomplished with density gradient centrifugation and/or antibody-coated beads.24

**TABLE 1 Suggested Whole-Blood Processing Practices**

<table>
<thead>
<tr>
<th>Step</th>
<th>Serum</th>
<th>Plasma and Buffy Coat</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection from whole blood</td>
<td>Use the same collection tubes for each sample to reduce changes in analytes23,85–88</td>
<td>Use the same collection tubes for each sample to reduce changes in analytes23,85–88</td>
<td>Sodium citrate or EDTA tubes are optimal90</td>
<td>PAXgene tubes</td>
</tr>
<tr>
<td>Time to centrifugation</td>
<td>30–60 min or longer if patient is treated with anticoagulants97</td>
<td>Immediately97</td>
<td>Immediately97</td>
<td>For the PAXgene tubes, sample must be incubated at RT* for a minimum of 2 h to ensure complete lysis and inactivation of ribonucleases before isolation90</td>
</tr>
<tr>
<td>Samples processed in &lt;30 min can retain cellular components that may influence downstream analysis97</td>
<td>Keep lag time before centrifugation constant to reduce sample variability97</td>
<td>Keep lag time before centrifugation constant to reduce sample variability97</td>
<td>RNA in PAXgene tube is stable for 72 h at RT, 4–6 d at 4°C90</td>
<td></td>
</tr>
<tr>
<td>Time to freeze</td>
<td>Immediately97</td>
<td>Immediately97</td>
<td>Process DNA before freezing to avoid hemolysis99</td>
<td>RNA in PAXgene tube is stable for up to 5 d at RT and up to 12 mo at −20°C100</td>
</tr>
<tr>
<td>Storage</td>
<td>−70°C minimum to maximize storage duration without changes in sample quality75,76</td>
<td>−70°C minimum to maximize storage duration without changes in sample quality75,76</td>
<td>Stable at 4°C for several weeks, at −20°C for months, and at −80°C for years11</td>
<td>−70°C minimum once RNA has been isolated75</td>
</tr>
<tr>
<td>Freeze-thaw</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity76</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity76</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity76</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity76</td>
</tr>
</tbody>
</table>

*RT = 20–25°C.
TABLE 2 Examples of Markers Artificially Altered by Coagulation, Anticoagulants, or Collection Techniques

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Examples of Biomarkers Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (due to coagulation process)</td>
<td>• VEGF is highly expressed in serum due to platelet secretion during clotting in both healthy patients95,96 and in those with disease97–99  • MMPs increase with clot accelerators due to amorphous silica or silicate salts (components of clot activators)100  • α2 Macroglobulin, BDNF, EGF, ENA-78, IL-8, PAI-1, and TIMP-1 are higher in serum samples from healthy patients compared with plasma samples using EDTA, sodium citrate, or heparin as anticoagulant101  • Lysophosphatidylcholines are higher in serum from healthy patients due to platelet release of phospholipases activated by thrombin102  • MMP-1, MMP-2, MMP-13, IL-4, IL-10, and growth factors increase in plasma isolated with EDTA103  • Neutrophil elastase, MMP-2, and MMP-9 increase due to isolation-dependent activation of neutrophils and mononuclear phagocytes104  • Tropomin-I and tropomin-T decrease in tubes with heparin due to binding105  • Glucose is lower due to fluid shifting from erythrocytes to plasma caused by anticoagulants, as compared with serum106  • Total cholesterol is lower due to fluid shifting from erythrocytes to plasma with EDTA, as compared with serum107  • HDL-C is higher in plasma due to EDTA, as compared with serum108  • Lysophosphatidylcholines are higher due to the storage of plasma sample at RT109  • FVIII levels decrease due to storage at RT before blood processing110  • VEGF levels increase with EDTA as anticoagulant110  • VEGF, MCP-1, eotaxin, and factor VII are increased in citrate and heparin plasma samples from healthy patients111</td>
</tr>
<tr>
<td>Plasma (due to the use of different anticoagulants or collection techniques)</td>
<td>• MMP-1, MMP-2, and TIMP-1 are increased in citrate and heparin plasma samples,34,112 plasma samples using EDTA, sodium citrate, or heparin as anticoagulant113  • Neutrophil elastase, MMP-2, and MMP-9 increase due to isolation-dependent activation of neutrophils and mononuclear phagocytes114  • Tropomin-I and tropomin-T decrease in tubes with heparin due to binding115  • Glucose is lower due to fluid shifting from erythrocytes to plasma caused by anticoagulants, as compared with serum116  • Total cholesterol is lower due to fluid shifting from erythrocytes to plasma with EDTA, as compared with serum117  • HDL-C is higher in plasma due to EDTA, as compared with serum118  • Lysophosphatidylcholines are higher due to the storage of plasma sample at RT119  • FVIII levels decrease due to storage at RT before blood processing120  • VEGF levels increase with EDTA as anticoagulant120  • VEGF, MCP-1, eotaxin, and factor VII are increased in citrate and heparin plasma samples from healthy patients121</td>
</tr>
</tbody>
</table>

BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; ENA-78, epithelial neutrophil-activating peptide 78; FVIII, factor VIII; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor 1; TIMP-1, tissue inhibitor of metalloproteinase 1; VEGF, vascular endothelial growth factor.

* RT = 20–25°C.

Influences cell metabolism and alterations of cell membrane integrity, resulting in release of metabolites and/or degraded products.20 Immunolabeling and flow cytometry should use whole blood to avoid centrifugation and washing steps, which can lead to artifactual cell activation.32 Flow cytometry assays should avoid blood taken through intravenous cannulae and should use a direct thrombin inhibitor as an anticoagulant, minimize and standardize time delays between blood collection and processing, and store fixed samples at 4°C before analysis.32 Tables 4, 5, and 6 summarize the effects produced by different anticoagulant or processing techniques on a variety of blood cell types.

ADDITIONAL TUBE ADDITIVES

Proteins can be degraded or undergo structural modifications due to enzymes naturally present in human blood, including proteases, peptidases, and phosphodiesterases. Protein degradation can be reduced by adding an enzyme inhibitor or inhibitors to the blood and by rapidly separating and freezing plasma. Blood can be collected into calcium chelators, such as citrate, which inhibits calcium-dependent proteases involved in the coagulation cascade. Calcium chelators can be used in combination with enzyme inhibitors.33 Clotting-related molecules are highly influenced by protease activity in blood or plasma samples,34–36 as are samples with activated, disrupted, or lysed neutrophils37 or mononuclear phagocytes38 that naturally release enzymes. Tubes containing calcium chelators and enzyme inhibitors are summarized in Table 3.

DETECTION OF IMMUNOGLOBULINS

Detection of infectious pathogens is often based on the determination of immunoglobulin antibodies in serum and plasma.39 Immunoglobulins can be stable at room temperature (RT) for days,25 but stability increases if serum aliquots are stored in dimethyl sulfoxide at −70°C.25 The detection of immunoglobulins is altered by blood surfactants that may lead to the loss of antibody from the solid phase, affecting immunoassays during antibody diagnostics.40,41 An alternative method for antibody diagnostics is the use of dried blood spots on either filter paper or Guthrie cards42–44; blood must be dried for several hours at RT and then stored in sealed plastic envelopes at −20°C.42–44

ISOLATION OF NUCLEIC ACIDS

Cell-free nucleic acids are present at low levels in the serum and plasma of healthy patients; however, illnesses result in a significant increase in nucleic acid blood levels.45–49 Sources of cell-free nucleic acid include the apoptosis/necrosis of normal or cancer cells, or from microorganisms such as bacteria or viruses.49 Therefore, the study of cell free nucleic acids in serum or plasma offers a valuable research opportunity in specific circumstances. Alternatively, DNA and RNA can be directly isolated from cellular
## TABLE 3 Commonly Used Blood Collection Tubes

<table>
<thead>
<tr>
<th>Tube Additive (Product)</th>
<th>Tube Description</th>
<th>Laboratory Use</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clot activator/gel (serum) BD: Gold top</td>
<td>General blood chemistry</td>
<td>Clotting time: 30 min</td>
<td>Rapid serum separation, decreases hemolysis&lt;sup&gt;100–111&lt;/sup&gt; Analysing instability when exposed to the gel barrier&lt;sup&gt;112&lt;/sup&gt; Gel barrier is unstable under extreme temperature&lt;sup&gt;21&lt;/sup&gt;</td>
</tr>
<tr>
<td>Silicone coated, clot activator (serum) BD: Red top</td>
<td>General blood chemistry, serology, and blood banking</td>
<td>Clotting time: 60 min</td>
<td></td>
</tr>
<tr>
<td>Thrombin-based clot activator (serum) BD: Orange top</td>
<td>Stat determinations in general blood chemistry</td>
<td>Clotting time: 5 min</td>
<td></td>
</tr>
<tr>
<td>Clot activator (serum) or K&lt;sub&gt;2&lt;/sub&gt;EDTA (plasma) BD: Royal blue top</td>
<td>Trace elements, toxicology, and nutritional-chemistry</td>
<td></td>
<td>Fluoride does not prevent loss of plasma glucose during the first 30–90 min after blood collection&lt;sup&gt;114&lt;/sup&gt; but does prevent the loss at later times by inhibiting endolase activity&lt;sup&gt;115&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaF (serum) or NaF and oxalate or EDTA (plasma) BD: Gray top</td>
<td>Glucose determinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium citrate (plasma) BD: Light blue top</td>
<td>Routine coagulation determination</td>
<td>Platelets are most stable in citrate anticoagulant&lt;sup&gt;23&lt;/sup&gt; High-quality DNA and RNA&lt;sup&gt;23,26&lt;/sup&gt; Produces high yield of lymphocytes for culture&lt;sup&gt;23,26&lt;/sup&gt; Can cause a dilution of the plasma</td>
<td></td>
</tr>
<tr>
<td>Heparin (plasma) BD: Green top</td>
<td>Plasma determinations in chemistry</td>
<td>Heparin binds to different cellular proteins, potentially interfering with downstream analysis&lt;sup&gt;25–27&lt;/sup&gt; Heparin inhibits T-cell proliferation and culture efficacy&lt;sup&gt;25,26&lt;/sup&gt; To measure UE, LFTs, cardiac enzymes, Ca&lt;sup&gt;2+&lt;/sup&gt;, Mg&lt;sup&gt;2+&lt;/sup&gt;, phosphate, uric acid, total protein, amylase, lipids, bone profile, troponin, iron status, and ACE&lt;sup&gt;116&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Heparin and gel (plasma) BD: Light green top</td>
<td>Plasma determinations in chemistry</td>
<td>Lead levels are more accurate with EDTA as anticoagulant than heparin&lt;sup&gt;117&lt;/sup&gt; Samples can undergo marked changes before centrifugation&lt;sup&gt;23&lt;/sup&gt; Lead levels are more accurate with EDTA as anticoagulant than heparin&lt;sup&gt;117&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA (plasma) BD: Lavender or pink top</td>
<td>Whole-blood hematology determinations</td>
<td>Good for DNA-based assays Can influence Mg&lt;sup&gt;2+&lt;/sup&gt; concentrations and interfere with cytogenetic analysis&lt;sup&gt;25&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA (plasma) BD: Tan top; PED: No</td>
<td>Lead determinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA and gel (plasma) BD: White top</td>
<td>Molecular diagnostic testing (such as, but not limited to, polymerase chain reaction and/or branched DNA amplification techniques)</td>
<td>A combination of a spray-dried anticoagulant and a gel material, which separates erythrocytes, granulocytes, lymphocytes, and monocytes from the supernatant&lt;sup&gt;118&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA and protein stabilizers (plasma) BD: P100 tubes</td>
<td>For protein preservation and isolation</td>
<td>Provides the anticoagulant activity of EDTA and enhanced stability of peptides and proteins&lt;sup&gt;100&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PPACK, aprotinin, and EDTA PED: No</td>
<td>For protein preservation and isolation</td>
<td>In stock or custom-made tubes with different additives and protein inhibitors according to the researcher’s needs</td>
<td></td>
</tr>
<tr>
<td>PPACK, aprotinin, and EDTA PED: No</td>
<td>For protein preservation and isolation</td>
<td>Compatible with proteomic analyses, including MALDI-TOF, LC-MS, 2D-PAGE, and immunoassays&lt;sup&gt;22&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA and DPP-IV, protease inhibitor cocktail (lyophilized) BD: P700 tubes</td>
<td>GLP-1 determination</td>
<td>Recovery and preservation of plasma GLP-1 is higher using P700 tubes compared with other tubes; the stability of GLP-1 is in the following order: P700 &gt; P100 &gt; EDTA &gt; heparin = citrate &gt; serum&lt;sup&gt;122&lt;/sup&gt; Compared with other tubes, the peptide GLP-1 was still detected after incubation for 96 h in P700&lt;sup&gt;122&lt;/sup&gt; The P800 tubes are compatible with proteomic analyses, including MALDI-MS, and immunoassays&lt;sup&gt;123&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;EDTA and proprietary cocktail of protease, esterase and DPP-IV inhibitors BD: P800 tubes</td>
<td>GLP-1, GIP, glucagon, and ghrelin determinations</td>
<td>Degradation of GLP-1 and GIP peptides were observed within 2–6 h in EDTA plasma, whereas P800 stabilized these peptides for 96 h. In EDTA, ghrelin half-life is 15.9 h, whereas in P800 its half-life is &gt;3 d&lt;sup&gt;123&lt;/sup&gt; The P800 tubes are compatible with proteomic analyses, including MALDI-MS, and immunoassays&lt;sup&gt;123&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; BD, BD Biosciences; DPP-IV, proprietary dipeptidyl peptidase IV, ELISA, enzyme-linked immunosorbent assay; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, Glucagon-like peptide 1; K<sub>2</sub>EDTA, dipotassium EDTA; LC-MS, liquid chromatography–mass spectrometry; LFT, liver function test; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; PPACK, D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone; 2D-PAGE, 2-dimensional polyacrylamide gel electrophoresis; PED, pediatrics; SCAT, special collection anticoagulant tubes; UE, Na, K, urea, creatinine.
fraction, often producing better yield and quality of nucleic acids than unprocessed whole blood. Potential effects produced by different anticoagulants and processing techniques on DNA and RNA isolation are summarized in Table 7.

### DNA Isolation

Proper practice is crucial to ensure quality DNA isolation. Commercial kits exist for DNA extraction from serum and plasma, which rely on similar methodologies and produce equivalent yields of DNA. The QiAamp Viral Spin Kit (Qiagen, Venlo, Netherlands), using silica-based membrane chemistry, is reported to produce high-yield DNA from plasma and serum. The ReliaPrep Blood gDNA Miniprep System, based on cellulose-based chemistry, and the Maxwell 16 LEV Blood DNA Kit, based on paramagnetic particle chemistry, provide all-in-one methodologies for DNA purification from either blood or buffy coat (Promega, Fitchburg, WI). The quality and yield of DNA can also be obtained inexpensively with traditional phenol/chloroform extraction procedures, but these extractions are time consuming and labor intensive. Tubes containing citrate or EDTA are optimal choices for the isolation of cell-free DNA. Alternatively, the PAXgene Blood DNA System is available for collection and stabilization of whole blood for isolation of genomic DNA.

### RNA Isolation

It is imperative to prevent degradation of RNA during collection, transport, storage, and processing. Commercial kits, using similar methodologies, are available for RNA isolation from blood or cellular fractions with similar efficiency. All kits are intended for easy collection, storage, and transport of blood while maintaining RNA stability, followed by the isolation and purification of RNA. Choices include the QiAamp UltraSens Virus Kit (Qiagen) for isolating mRNA from plasma or several alternative kits depending on the type of isolation method, the preparation time, and the type of stabilization reagents (RNA later; Tempus, PAXgene, EDTA, citrate or heparin tubes; Life Technologies, Carlsbad, CA). BD Vacutainer CPT cell preparation tubes are also available to isolate RNA.

### TIMING OF SAMPLE PROCESSING

Blood components are labile in nature, and their integrity requires timely processing. For serum separations, the time allowed for samples to clot is critical, although it varies between samples on the basis of intrinsic clotting factor levels and clinical exposure to anticoagulants. Incubation times longer than required for adequate clotting may result in cell lysis, thereby altering analyte levels. Higher molecular weight proteins, such as lipoproteins, are more sensitive to prolonged clotting time, resulting in potential variability. Plasma samples do not require time to clot but require rapid processing to limit biomarker alterations. Cytokine concentrations in plasma change in <2 hours when maintained at RT. In general, nucleic acids should be processed quickly to optimize yield and integrity.

Ideally, blood should be processed and frozen rapidly after collection. Placing samples on ice before processing can improve sample preservation; however, samples should still be processed within 30 to 60 minutes to maintain integrity. Furthermore, the use of ice or cold packs in the transport of blood from the clinical setting to the laboratory can reduce protein degradation. Temperature consistency of samples during processing is imperative to maintain sample quality. It is also important to maintain consistent lag times between sample collections and processing.
TABLE 5 Effects Produced by Anticoagulants or Processing Techniques on White Blood Cell Isolation

<table>
<thead>
<tr>
<th>Leukocytes</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect on cell morphology</td>
<td>EDTA: excess EDTA causes leukocytes membrane damage⁷⁶</td>
</tr>
<tr>
<td>Effect on cell morphology</td>
<td>EGTA and oxalate: produce lower yield of cells⁷⁰</td>
</tr>
<tr>
<td>PMNs</td>
<td>EDTA: vacuolization of PMNs occurred after 3–4 h of storage at RT* and increased after 6 h⁷³, no or minimal changes were found when samples were stored at 4°C⁷²</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>EDTA: results in an increase in the number of PMNs isolated and a decrease in PMN activation, as compared with sodium citrate or heparin.⁵³ PMN count remains stable for ≥3 d with EDTA when whole blood is stored at RT¹⁴</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Heparin and sodium citrate: decreases the efficiency of PMN recovery¹⁵</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Heparin: increases PMN activation due to stimulation of adhesion molecules such as integrins⁷⁶</td>
</tr>
</tbody>
</table>

PBMC, peripheral blood mononuclear cell; PMN, polymorphonuclear neutrophil.
* RT = 20–25°C.

because lag-time differences produce variability between samples⁶⁹,⁷⁰

**STORAGE TEMPERATURE**

Storage temperatures depend on the nature of the analyte that is being preserved. Although DNA isolated from blood samples is stable at 4°C for several weeks, at −20°C for months, and at −80°C for years.⁷¹ RNA degrades quickly at temperatures higher than −80°C.⁷⁵ Proteins and other soluble biomarkers have varied stability; immunoglobulins are stable at RT for days,⁷⁵ whereas other proteins are exceedingly labile and must be frozen at −80°C.⁶⁸,⁷² Viable cells can typically survive at RT for ≤48 hours, but they must be eventu-

ally cultured at physiologic temperatures or frozen in liquid nitrogen with a cryoprotective agent for long-term preservation.⁷３,⁷⁴

In general, −80°C is usually sufficient to preserve a broad range of molecules. Protein profiling is preserved for ≥4 years of storage at −70°C.⁷⁵,⁷⁶ Nonetheless, the long-term stability of many molecules is poorly understood, and long-term storage of samples should be limited to <4 years from sample procurement.

**FREEZE-THAW OF SAMPLES**

Although frozen samples are stable for long-term storage, they are especially vulnerable to freeze-thaw cycles. Freeze-thawing results in the formation of ice crystals, which can cause significant destruction to biological molecules. A single freeze-thaw at −70°C decreases the yield of DNA by 25%,⁷⁷ and multiple freeze-thaw cycles can influence many other molecules.⁶⁷,⁷⁶,⁷⁸,⁷⁹

Freeze-thaw damage is limited by aliquoting samples into small volumes that are sufficient for a single experimental, and then flash-freezing samples with either liquid nitrogen or by cooling ethanol on dry ice for tube submersion.⁸⁰

**SHIPMENT**

Blood specimens, with or without infectious agents, are considered dangerous goods and must be properly classified, identified, packed, labeled, and handled for shipping.⁸¹ Various agencies have published rules and regulations regarding the transportation of medical specimens on the basis of the associated risks and safety standards,⁸² and they also provide training and expert advice on dangerous goods transportation. International Air Transport Association (IATA) guidelines for transport of dangerous goods⁸³ as well as local laboratory protocols must be followed.

Specimens must be kept within a specific temperature range during shipment to protect sample integrity. Temperature maintenance and packaging depend on the nature of the sample and the proposed analysis. Samples can be shipped at RT (20–30°C), refrigerated (2–8°C), or frozen (−20°C, −70°C, −150°C). Shipping boxes and packs to maintain specific temperatures, in addition to absorbent strips, bubble wrap, and polypropylene secondary-pressure vessels with O-rings for leak-proof packaging, are available. Samples that should be maintained below −70°C require dry
ice in the shipping box, whereas a liquid nitrogen dry shipper must be used for samples shipped at $-150^\circ$C. Shipping of frozen specimens with dry ice requires "UN" dry ice labeling on the box, including the name and addresses of shipper and consignee, and the weight of the dry ice must be marked near the class 9 diamond-shaped hazard label.

Specimens are designated category "A" if they are blood samples or cultures known to contain certain infectious agents or category "B" if they are blood specimens typical of clinical diagnostic tests. Specific leak-proof containers for specimens classified as A or B are available commercially.

Category A specimens must be shipped in a certified container according to IATA regulations. Several commercial laboratories offer a variety of infectious certified containers for frozen, refrigerated, or ambient specimens that include secondary and outer packages and in most cases contain the appropriate hazard labels. Category A specimens are assigned to UN2814-Infectious Substance, affecting humans. The assignment to UN2814 is based on the medical history of the patient or by his or her symptoms. If it is not clear whether a pathogen falls within this category, it must be transported labeled as a Category A Infectious Substance. To help with the assignment, the IATA has created a table with examples of different pathogens that meet the criteria of category A. For packing, a category A specimen is also shipped in a triple package but according to Packing Instruction 620. A box containing a category A substance must have affixed to it an Infectious Substance class 6 hazard label; the name, address, and telephone numbers of shipper and consignee; as well as a Shipper’s Declaration for Dangerous Goods. If the substance is a liquid, it must have orientation arrows on the outside of the box or the words THIS END UP to specify the correct orientation of the container inside. If a package contains $>50$ mL or $50$ g of a category A infectious material, then a Cargo Aircraft Only label is required.

For category B specimens, biohazard color-coded temperature bags and insulated Styrofoam shipping containers are available to maintain the proper temperature. Styrofoam shipping containers should be used with dry ice, frozen cool packs should be used for refrigerated samples, and RT cool packs should be used for shipping at 20 to $30^\circ$C. IATA regulations require shipping of specimen category B in a triple package according to Packing Instruction 650. The triple packaging consists of the following: (1) a leak-proof primary receptacle (eg, cryovials or Vacutainer tubes), (2) a leak-proof secondary packaging (eg, sealed Styrofoam container [minimum of 1-in thickness], sealed plastic bag, plastic canister, or screw-cap can), and (3) an outer rigid packaging (eg, corrugated fiberboard or wood boxes, rigid cooler, rigid plastic container) of adequate strength for its capacity and mass. Each completed package must

### TABLE 6 Effects Produced by Anticoagulants or Processing Techniques on Platelet Isolation

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td></td>
</tr>
<tr>
<td>Effect on cell morphology</td>
<td>- EDTA induces platelet swelling, changes in size distribution, and accelerates shape changes.137</td>
</tr>
<tr>
<td>Effect on cell chemistry</td>
<td>- Heparin: increases the number of platelet-monocyte aggregates compared with PPACK, sodium citrate, and EDTA.25</td>
</tr>
<tr>
<td>Effect on sample handling or collection</td>
<td>- EDTA and citrate: differentiates more accurately the number of single cells that are present in the sample from clusters by dispersing and decreasing the number of reversible platelet-neutrophil aggregates.25 or the number of platelet-monocyte aggregates compared with PPACK,25 or compared with hirudin and heparin.154</td>
</tr>
<tr>
<td>Effect on cell chemistry and on sample handling or collection</td>
<td>- EDTA and EGTA: also reduce the number of platelet-leukocyte aggregates32</td>
</tr>
<tr>
<td></td>
<td>- EDTA decreases viability of platelets compared with acid citrate dextrose137</td>
</tr>
<tr>
<td></td>
<td>- PPACK: the preferred anticoagulant for platelet isolation due to less platelet activation25</td>
</tr>
<tr>
<td></td>
<td>- Citrate: the preferred anticoagulant in the event of a predicted delay to immunostaining and fixation,25 but can produces cell aggregates.37</td>
</tr>
<tr>
<td></td>
<td>- Platelet-monocyte aggregates remain longer in peripheral blood, and they can be quantified by flow cytometric analysis.32</td>
</tr>
<tr>
<td></td>
<td>- BAPA (dual inhibitor of factor Xa and thrombin): recommended when delays are expected between blood collection by venipuncture and transportation to remote locations; maintains stable platelet function response (IPA and ATP secretion) and cell dense granule secretion up to 48 h at RT compared with sodium citrate.139</td>
</tr>
<tr>
<td></td>
<td>- Platelet-monocyte aggregates increase in sample from intravenous cannulae compared with sample obtained by venipuncture.37</td>
</tr>
<tr>
<td></td>
<td>- Citrate-phosphate-dextrose: concentrations of lyssolecithins (lysoPCs, compounds derived from phosphatidylcholines) increase in these platelets stored for 5–7 d compared with the fresh platelet sample.126</td>
</tr>
</tbody>
</table>

ATP, adenosine-5’-triphosphate; BAPA, benzylsulfonyl-D-Arg-pro-4-amidinobenzylamide; EGTA, ethylene glycol tetraacetic acid; IPA, Impedance platelet aggregation; PPACK, D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone.  

a RT = 20–25°C.
be capable of passing the IATA drop-test from a height of not less than 1.2 m. For liquids, absorbent material (e.g., cellulose wadding, cotton balls, superabsorbent packets, and/or paper towels) in sufficient quantity to absorb the entire contents in the package must be placed between the primary receptacle or receptacles and the secondary packaging. Category B specimens are assigned to UN3373 with a diamond-shaped hazard label, a proper shipping name of biological substance “Category B,” and Packing Instruction 650.

Specimens derived from patients that are unlikely to cause disease or do not contain infectious substances are not subject to regulations unless they meet a criterion of inclusion, and they must be in a triple package according to Packing Instruction 650 and marked with the words “Exempt human specimens.”

**CONCLUSIONS**

This review summarizes pertinent issues in the collection, processing, shipping, and storage of blood samples for use in child health research studies. Importantly, there is no single ideal method for the preservation and storage of all types of samples. It is advisable to consider each analyte of interest unique and to investigate its stability and integrity before the procurement of a large number of samples. A pilot study in which control blood samples are processed and markers of interest are investigated is ideal, ensuring that the standard operating protocols are sufficient for the preservation of samples and limiting the potential for quality control issues.

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Carolina Gillio-Meina, Gediminas Cepinskas, Erin L. Cecchini and Douglas D. Fraser
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