

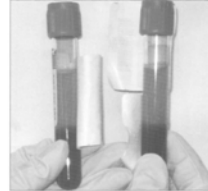
Pre-Analytic Issues in Laboratory Medicine

Daniel J. Fink, MD, MPH
Director, Core Laboratory
New York Presbyterian hospital
Columbia University Medical Center

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Pre-Analytic or Extra-Terrestrial?

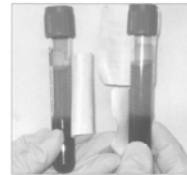
TRANSFUSION MEDICINE ILLUSTRATED



Pre-Analytic Issues in Laboratory Medicine

The lab made a mistake!

Iatrogenic Green Plasma due to Isosulfan Blue Dye



*Julie L. Housenorth, Jodi A. Parks, Evangeline A. Miguel, Leo J. McCarthy, and
Constance EM. Danielson*

A 50-year-old woman presented for radical vulvectomy and bilateral groin lymphadenectomy secondary to Stage II squamous cell carcinoma of the vulva. A request for a type and screen was sent to the blood bank. The plasma was a striking overall green color, and a newly collected specimen received about 6.5 hour later was unchanged in appearance (see figure). Further investigation disclosed that during surgery the leading edges of the patient's tumor were injected with 5-ml of isosulfan blue (lymphazurin). The patient also received IV heparin 5000 units. The dye was used to enable precise identification of sentinel nodes by afferent blue channels to facilitate a bilateral lymphadenectomy.

Pre-Analytic Issues in Laboratory Medicine

Phases of Testing

- Pre-Analytical
 - Test Ordering, Specimen Collection, Specimen Handling
- Analytical
 - Test Performance, Quality Control, Result Review
- Post-Analytical
 - Result handling, Result Communication, Result Interpretation

Why are Pre-Analytical Issues Important?

- Abnormal test results usually are attributed to disease. This is not always the case.
- Important considerations in interpreting laboratory results include preanalytical and biological variation.
- **Preanalytical** variation is due to factors external to the patient affecting laboratory specimens prior to testing. Being aware of these factors and following proper procedures will minimize these effects, allowing for more reproducible and accurate results.
- **Biological Variation** is due to factors inherent to the patient that may or may not be controllable

Patient Identification

- It is important to identify a patient properly so that blood is collected from the correct person.
- .1% - 1% of specimens are from the wrong patient.
- Two patient identifiers should be used.
- Hospital inpatients should be wearing an identification band which should be checked before the blood is drawn. Blood should not be drawn from a patient without a band.
- Test forms should be compared to the inpatient's wrist bracelet or verbally confirmed with an outpatient.

Biologic Variability

- Age, sex, race
 - Alkaline phosphatase in children
 - Neutrophil counts in neonates
 - Creatinine, CK in females, blacks
- Diurnal variation
 - Glucose values obtained during an oral glucose tolerance test tend to be higher when the test is performed in the afternoon than when the test is performed in the morning.
- Diet
 - High-protein and high-purine diet increase levels of uric acid, urea, and ammonia in blood compared with vegetarians
- Smoking
 - Long-term smoking increases carboxyhemoglobin, hemoglobin, RBC, WBC and MCV values;
 - WBC level related to number of packs smoked

JCAHO 2005 Laboratory Services National Patient Safety Goals

Goal: Improve the accuracy of patient identification.

Use at least two patient identifiers (neither to be the patient's location) whenever collecting laboratory samples or administering medications or blood products,

Use two identifiers to label sample collection containers in the presence of the patient.

Test Ordering

- Order the relevant test
- Know when to order
- Know how often to order
- Know how the results will be used
- Consult with the laboratory director

Avoiding Specimen Labeling Errors

- Match patient identification on the labels to the order and patient ID using two identifiers
- Draw and label specimens at the bedside, one patient at a time
- Affix proper specimen labels to the collection tubes immediately after specimen collection (do not place drawn tubes in a cup or emesis basin and proceed to another task before affixing labels)
- Do not draw extra unlabeled tubes
- The person who collects the specimen should label the specimen
- Avoid secondary labeling where the specimen is labeled by hand and then printed labels are attached later.

Specimen Collection

- Postural Effects
- Collection Tubes and Additives
- Affect of Tourniquet Time
- Collection from IVs and Catheters
- Volume effects
- Avoiding Clots
- Avoiding Hemolysis

Plastic versus Glass Tubes

- Plastic tubes have replaced glass tubes for most applications
- Less breakage, cheaper and lower weight
- Clot activators needed to be added to serum tubes
- Give clinically equivalent results for almost all analytes

Postural Effects

- Change in posture from supine to erect or sitting causes a shift in fluid from the intravascular to the interstitial space of about 12%.
- An increase of 5% to 15% is seen for most cellular and macromolecular analytes when specimens are collected sitting as compared to supine.
- Conversely, moving from upright to supine can have a dilutional effect owing to an increase in plasma volume
- The effect of postural change is accentuated in patients with a tendency to edema.

Collection Tube Additives

- Heparin
- EDTA
- Citrate
- NaF + K Oxalate
- Clot Activator
- Serum Separator

Postural Effects

- Albumin levels are higher among healthy outpatients as compared to supine healthy hospitalized subjects
- Glucose (and other small molecules) move freely between the interstitial space and the circulation and are least affected by posture during blood specimen collection.
- While the free fraction of a metabolite, drug, hormone, or metal ion is not subject to postural variation, the fraction bound to proteins is affected by posture. Thus, bilirubin bound to albumin and calcium bound to albumin are affected by postural changes.
- A change from upright to supine can reduce (after 5 minutes) cholesterol level by 10% and triglyceride by 12%.

Heparin

- Used to collect whole blood or plasma
- Binds to anti-thrombin III to inhibit Xa, IXa, and thrombin
- Nominal concentration of 12 – 30 U/mL
- Heparin binds calcium so ionized calcium must be collected using “Calcium Titrated” or “Electrolyte Balanced” heparin

EDTA

- K₂EDTA is used to collect whole blood for hematology studies and plasma for analytes with heparin interference
- Acts by binding calcium
- Nominal concentration of 1.5 mg/mL
- Recent move to K₂EDTA from K₃EDTA for hematology to reduce affect on RBC parameters

NaF + K Oxalate

- NaF + K Oxalate is used to poison glycolytic pathway and to anti-coagulate specimens for glucose testing
- Glucose decreases by 5 – 7% per hour in specimens from adults and by up to 24% per hour in specimens from neonates and with very high white counts
- Delay in action leads to approximately 9 mg/dL loss over the first 3 hours after collection
- Causes a great deal of hemolysis and not suitable for other testing

EDTA Effects

- EDTA is hyperosmolar causing cell shrinkage but the low pH of EDTA counterbalances this effect by causing K (and water) to flow into cells.
- EDTA may cause platelet clumping and platelet satellitism that may be the result of changes in the membrane structure occurring when the calcium ion is removed by the chelating agent, allowing the binding of pre-formed antibodies.
- Sodium citrate tubes are sometimes collected to obtain more accurate platelet counts.

Clot Activator

- In vitro activation of clotting system to enhance clot formation
- Tubes contain a silica clot activator attached to the wall with a silicone surfactant
- Requires inversion of tube for optimal function
- Requires 15 to 30 minutes (instead of 1 hour) to complete clot formation.

Citrate

- Citrate is most often used for collection of coagulation tests
- Acts by binding calcium
- Nominal concentration of 3.2% (mol/L)
- Recent move to 3.2% from 3.8% to get more consistent results for Prothrombin Time, particularly for more sensitive reagents
- Tubes must be properly filled to within +/- 10 percent of assigned collection volume

Serum Separator Gel

- Polymer gel with specific gravity between that of serum (or plasma) and cells
- Migrates and forms a barrier during centrifugation
- Certain analytes and therapeutic drugs may bind to gel over time

Specialized Additives for Blood Collection

Glycolytic inhibitors

- NaF (2.5 mg/mL of blood with EDTA [1 mg/mL] or potassium oxalate [2 mg/mL])
- Lithium iodoacetate (0.5 mg/mL of blood) alone or in combination with lithium heparin (14.3 U/mL of blood)
- Sodium fluoride and lithium iodoacetate require 3 h to fully become effective
- Adding mannose (3 mg/mL of blood) to NaF achieves efficient glycolytic inhibition; Mannose, causes concentration-dependent interference
- Maintaining blood pH at 5.3-5.9 with mixture of citric acid, trisodium citrate, disodium EDTA, and NaF stabilizes glucose

Cell stabilizers

- Acid-citrate dextrose A and B formulations; B formulation has greater amounts of dextrose available to metabolizing cells
- Citrate, theophylline, adenosine, dipyridamole mixture minimizes in vitro platelet activation

Proteolytic enzyme inhibitors

- EDTA (1.5 mg/mL of blood) with aprotinin (500-2,000 KIU/mL) stabilizes several polypeptide hormones
- Aprotinin also can be used with lithium heparin (14.3 U/mL)
- A mixture of EDTA, aprotinin, leupeptin, and pepstatin stabilizes parathyroid hormone-related protein

Catecholamine stabilizers

- An antioxidant such as glutathione, sodium metabisulfite, or ascorbic acid at a concentration of 1.5 mg/mL is used with egtaic acid, EDTA, or heparin
- For urine, use 5 mL of a 6 Molar HCl per liter of urine or 250 mg each of EDTA and sodium metabisulfite per liter of urine

Duration of Tourniquet Application

- Application of the tourniquet for >1 minute can result in hemoconcentration, causing an increase in the concentration of large molecules (e.g. serum proteins) that are unable to pass through the capillary wall.
- Total protein, iron, total lipids and cholesterol increase from 5%-7%, bilirubin increases 8% and AST 9%
- Prolonged tourniquet application also promotes anaerobic glycolysis resulting in an increase in plasma lactate, a reduction in blood pH, and an increase in blood potassium.
- Repeated fist clenching during phlebotomy can also cause a 1 – 2 mEq/L increase in potassium.

Collection Tube Additives

- Heparin
- EDTA
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- NaF + K Oxalate
- Clot Activator
- Serum Separator

Collection from IVs and Catheters

- Blood should not be collected proximal to an IV site but preferably from the other arm
- Heparin may contaminate specimens collected from central lines unless flushed out with blood
- High glucose and/or low electrolyte values may result from collecting blood an IV or Central Line
- If questionable results are obtained from a sample collected through a catheter, the results should be verified by sending a new sample drawn from a different site

Order of Draw

Closure Color	Collection Tube	Mix by Inverting
BD Vacutainer® Blood Collection Tubes (glass or plastic)		
	• Blood Cultures - SPS	8 to 10 times
	• Citrate Tube*	3 to 4 times
	• BD Vacutainer® SST® Gel Separator Tube	5 times
	• Serum Tube (glass or plastic)	5 times (plastic) none (glass)
	• Heparin Tube	8 to 10 times
	• BD Vacutainer® PST® Gel Separator Tube With Heparin	8 to 10 times
	• EDTA Tube	8 to 10 times
	• Fluoride (glucose) Tube	8 to 10 times

Collection from IVs and Catheters

- If a syringe is used, a small volume (<=10 mL) syringe is recommended so that clotting in the syringe during phlebotomy is avoided.
- If samples must be obtained from a catheter, heparin contamination and dilution must be avoided. The line should be flushed with 5 mL of saline and the first 5 mL of blood or six dead space volumes of the catheter discarded.

Example of Contamination with IV Fluid

TEST	RESULTS		REFERENCE INTERVAL	UNITS
	Yesterday	Today		
Sodium	131	108	135-145	mmol/L
Potassium	4.0	3.0	3.5-5.0	mmol/L
Chloride	87	73	98-110	mmol/L
Bicarbonate	26	23	22-32	mmol/L
Urea	44.8	38.4	10-20	mg/dL
Creatinine	1.5	1.3	0.7-1.3	mg/dL
Glucose	108	149	65-110	mg/dL
Protein	7.9	6.1	6.0-8.0	g/dL
Albumin	3.6	2.8	3.5-5.0	g/dL

Avoiding Clots

- Use a sufficient amount of the correct anticoagulant
- Mix specimen thoroughly after collection
- Transfer immediately from syringe to tube
- Do not overfill tubes

Syringe Collection

- Visual hemolysis was found in 19% of specimens drawn by syringe, compared to 3% when drawn by an evacuated tube system.
- Also, 11% of syringe-collected EDTA samples exhibited clots
- If a syringe is used, the following can reduce the incidence of hemolysis:
 - Pump the plunger 2-3 times prior to collection to loosen the plunger.
 - Use a 3-10 mL syringe
 - Ensure that the speed of aspiration does not exceed 1mL of air space during collection. Excessive aspiration forces cause hemolysis.
 - Transfer the blood to the tubes immediately.
 - Fill tube by vacuum only. NEVER push down on the plunger; this increases the force of the blood flow, creating a high degree of red blood cell trauma.
 - Use a blood transfer device to transfer syringe-collected blood into a tube. It will enhance safety and improve specimen quality.

Avoiding Hemolysis

- Allow alcohol to dry before collection
- Use a larger bore needle
- Mix gently
- Avoid syringe collection if possible
- Avoid collection from IVs and catheters
- Draw slowly when collecting with syringes or from catheters
- Transport to lab and centrifuge in a timely fashion

Collection Volume

- Overfilled tubes
- Under filled coagulation tube
- Under filled hematology tube
- Under filling occurs because:
 - Tube was removed too quickly
 - Tube slips back from vacutainer needle
 - Air drawn in from butterfly or connector tubing

Arterial Blood Gases

- Avoid air contamination from a bubble or uncapped specimen
- Delay in analysis can cause high pO₂ to fall or low pO₂ to rise
- Analyze within 30 minutes or place on ice and analyze within 1 hour.
- Ca²⁺ binding by heparin can be minimized by using either of the following:
 - (1) A final concentration of sodium or lithium heparinate of 15 IU/ml blood or less
 - (2) Calcium titrated heparin with a final concentration of less than 50 IU/ml blood.
- Heparin Dilution effect can be avoided by use of dry heparin
- Roll specimen to mix heparin and reduce clots

Specimen Transport

- Specimens should be delivered to the laboratory promptly after collection
- Specimens should not be placed on ice unless specified by the laboratory
- Pneumatic tube transport does not affect analytical results

Primary Causes of Hemolysis

- Incomplete drying of skin after cleaning with alcohol
- Vigorous suction with syringe
- Inappropriate (too small) needle with syringe or vacutainer
- Forcing blood from syringe into tube when it has started to clot
- Shaking of tube instead of gentle agitation or inversion
- Inadequate packing in pneumatic tube container during transport to the laboratory to prevent shaking
- Prolonged contact of plasma or serum with cells
- Chilling (or freezing) of whole blood specimens
- For skin puncture specimens, squeezing tissues too hard during collection

Discussion

Anticoagulants for Blood Collection

- Heparin
- Unfractionated heparin molecular mass 3-30 kd (mean, 14 kd), as lithium, sodium, or ammonium salts; lithium heparin preferred for plasma chemistry testing; nominal amount, 14.3 U/mL of blood
- For ionized calcium, heparin in blood gas syringe can be calcium-titrated, electrolyte-balanced, or zinc lithium-titrated
- EDTA: salts of EDTA generally used for routine hematology testing (dipotassium or tripotassium EDTA, disodium EDTA); nominal amount, EDTA, 1.5 mg/mL of blood
- Citrate: generally used for routine coagulation testing; trisodium citrate-dihydrate 3.2% (0.109 mol/L)

Essentials of phlebotomy training for medical students

- Patient identification
- Completion of requisition form
- Labeling of specimen tubes – content and placement on tube
- Collection of multiple tubes vs. single tube
- Timing of specimen collection in relation to time of day and drug intake
- Posture
- Relation to meals
- Sites for collection
- Use of tourniquet
- Skin preparation
- Collection technique
- Appropriate tubes for ordered tests
- Order of collection of tubes
- Disposal of syringes, needles

Hematology Physiological Variables

- A number of physiological variables can be associated with certain patient factors, such as age. For example, at birth red blood cell count (RBC) and hemoglobin values are significantly higher than they are in adults, due to the relatively low levels of oxygen *in utero*. Within the first few months of life they fall substantially and continue to decrease and level out to adult values at about the age of fifteen. In addition, lymphocyte counts change throughout life; they are highest in children and lowest in the elderly.
- Smoking, high altitude, patient stance, exercise and pregnancy are some additional patient-related variables over which the lab has no control; yet, they may affect patient test results.¹⁰
- Additionally, white blood cell (WBC) and platelet counts can be affected by cryoglobulins. When blood specimens cool from body temperature to room temperature, the cryoglobulins aggregate and may be falsely identified as platelets and/or WBCs by the hematology analyzer. Cold agglutinins have been known to cause spurious reporting of macrocytosis and decreased RBC counts. Some automated instruments may also report falsely high WBC counts and platelet counts.³ The blood smear may show agglutination of RBCs.
- Platelet satellitism is a phenomenon that only occurs in EDTA anticoagulated blood. This is due to EDTA-dependent IgG autoantibodies and occurs at room temperature. When platelet satellitism is present, there may be a false elevation of cell counts.

<h2>Fibrin Interference</h2>
<ul style="list-style-type: none"> Residual fibrin, long recognized as a possible interferent in the clinical laboratory, may be present as a result of improper specimen handling during and after collection. It can be present in primary tube samples either as a visible clot, which may physically occlude the instrument sample probe or, more insidiously, as an invisible microfibrin or as strands. Fibrin strands, though invisible, may directly affect some assays, especially immunoassays.²⁻⁵ Unlike interference from heterophilic antibodies or rheumatoid factor, fibrin interference is usually not reproducible and disappears with time as the fibrin settles out of the sample. Care taken during the preanalytical phase can help to reduce the presence of fibrin strands in the processed specimen. Important considerations in the preanalytical phase that can have an effect on fibrin formation are shown in Table 2. <p> Table 2. Preanalytical phase considerations that can affect fibrin strand formation. </p> <ul style="list-style-type: none"> Recognition of disease or therapy that may affect clotting time Selection of the appropriate tube type Collection sequence when multiple tubes are collected

<h2>Specimen Collection Variables</h2>
<p>Tourniquet application time</p> <p>>1 minute increase in concentration of large molecules; for total cholesterol level, 5% increase at 2 minutes; up to 15% increase at 5 minutes</p> <p>Decreased pH; increased potassium, lactate, ionized calcium, and magnesium levels with increased application time</p> <p>Repeated fist clenching can increase potassium by 1-2 mmol/L, up to 2.7 mmol/L</p> <p>Infusion</p> <p>Spuriously increased blood glucose level if specimen obtained from arm receiving glucose infusion</p> <p>Waiting times before blood specimen collection: subjects receiving fat emulsion, 8 h; carbohydrate, protein, or electrolytes, 1 h</p> <p>Extent of hemolysis related to age of transfused blood</p> <p>Exercise</p> <p>Strenuous exercise increases total CK and CK-MB; CK-MB as percentage of total CK normal; decreased haptoglobin value due to intravascular hemolysis</p> <p>Individual variability in CK related to extent of exercise training</p> <p>Mitochondrial size and number increase with vigorous training; mitochondrial CK increases to >8% of total CK</p> <p>Increased epinephrine, norepinephrine, glucagon, cortisol, corticotropin, and growth hormone levels; decreased insulin level</p> <p>Increased serum and decreased urine uric acid levels due to increased lactate level</p> <p>Increased apolipoprotein AI and HDL-C levels; decreased LDL-C, apolipoprotein B, and triglyceride levels with long-term strenuous exercise or brisk walking</p>

<h2>Specimen Collection</h2>
<ul style="list-style-type: none"> Site Preparation: Prior to venipuncture, the site should be cleansed with alcohol. Before performing the venipuncture, the alcohol should be allowed to air dry. This will help to ensure that the specimen is not contaminated with alcohol, as this can lead to hemolysis. Hemolysis can result in the spurious elevation of such analytes as potassium, lactate dehydrogenase (LD), iron and magnesium in the chemistry lab. Proper Venipuncture Technique: During phlebotomy, avoid probing to find the vein and achieve blood flow. Excessive probing and/or "fishing" to find a vein can result in a poor quality sample, including hemolysis. Order of Draw: Following the correct order of draw during venipuncture will help to ensure accurate test results. The BD and CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS)

<p>Specimens for therapeutic drug monitoring (TDM) should be obtained after a steady therapeutic concentration of drug or steady state is achieved in the blood. A blood sample obtained just before administering a drug dose after a steady-state level is reached will reflect the trough level or the lowest concentration to be obtained with the established drug dose. If only 1 blood sample is to be collected for TDM, it is preferred that it reflect the trough level. Blood samples obtained when the drug concentration is at its maximum, will, of course, reflect the peak level. Peak-level specimens need to be obtained within 1 to 2 hours after intramuscular administration of the drug, 15 to 30 minutes after intravenous administration, or 1 to 5 hours after oral administration.</p> <p>Timing of specimen collection for TDM, however, depends on the rate of distribution of the specific drug. If the drug is infused intra-venously, approximately 1 to 2 hours after the completion of infusion are needed for the distribution phase to be completed, with exceptions such as digoxin and digitoxin, which need 6 to 8 hours for the completion of the distribution phase. In general, since the rate of oral drug absorption differs from person to person, blood specimens obtained for TDM usually are timed to reflect trough levels. For the monitoring of certain drugs, such as theophylline, antibiotics, and antiarrhythmic drugs, it may be necessary and useful to measure peak levels after intravenous administration.</p>

<h2>Specimen Collection Variables</h2>						
<table> <tr> <th>Variable/Effects</th></tr> <tr> <td> Fasting 12-h overnight fast recommended, since increase in triglycerides persist up to 9 h after fatty meal After 48-h fast, 240% increase in bilirubin level; decreased levels of prealbumin, albumin, and C₃ Effects dependent on body mass </td></tr> <tr> <td> Time Standardize time to rule out diurnal effect Therapeutic drug monitoring trough vs peak levels; timing regimens </td></tr> <tr> <td> Urine, timed overnight or first morning specimen concentrated, ideal for sediment enumeration 24-hour sample for clearance measurements and for analytes with diurnal variation Random or spot urine generally suitable But, sensitivity to detect slight increases in numbers of cells and casts and glucose and protein levels affected by dilution </td></tr> <tr> <td> Posture In general, 5%-15% increase for most cellular and large molecules (eg, albumin) in sitting posture Increase in upright to supine has dilutional effect: 10% and 12% decreases in cholesterol and triglyceride levels, respectively Change in posture has no effect on free drug and small molecules </td></tr> <tr> <td> Postural effect substantial in patients with reduced plasma volume (eg, congestive heart failure, cirrhosis) Increased aldosterone, norepinephrine, epinephrine, renin, and atrial natriuretic peptide levels </td></tr> </table>	Variable/Effects	Fasting 12-h overnight fast recommended, since increase in triglycerides persist up to 9 h after fatty meal After 48-h fast, 240% increase in bilirubin level; decreased levels of prealbumin, albumin, and C ₃ Effects dependent on body mass	Time Standardize time to rule out diurnal effect Therapeutic drug monitoring trough vs peak levels; timing regimens	Urine , timed overnight or first morning specimen concentrated, ideal for sediment enumeration 24-hour sample for clearance measurements and for analytes with diurnal variation Random or spot urine generally suitable But, sensitivity to detect slight increases in numbers of cells and casts and glucose and protein levels affected by dilution	Posture In general, 5%-15% increase for most cellular and large molecules (eg, albumin) in sitting posture Increase in upright to supine has dilutional effect: 10% and 12% decreases in cholesterol and triglyceride levels, respectively Change in posture has no effect on free drug and small molecules	Postural effect substantial in patients with reduced plasma volume (eg, congestive heart failure, cirrhosis) Increased aldosterone, norepinephrine, epinephrine, renin, and atrial natriuretic peptide levels
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<h2>Specimen Processing</h2>
<ul style="list-style-type: none"> Proper Tube Handling and Specimen Processing: Serum and plasma tubes each have their own special handling requirements. Serum Samples Serum specimens need to clot completely prior to centrifugation and processing. Blood specimens collected in tubes with a clot activator should be allowed to clot for 30 minutes to ensure complete clot formation; tubes without an additive need up to 60 minutes for Blood from patients who are receiving anticoagulant therapy may take longer to clot. Tubes should be allowed to clot at room temperature, upright in a test tube rack, with the closures on the tubes. Spinning the tube too soon may result in a gelatinous and/or fibrous serum sample that will require re-spinning. Plasma Samples do not require clotting prior to centrifugation. This allows the tube of blood to be drawn, mixed and centrifuged immediately, resulting in a quicker turn-around-time for test results. Centrifugation: The next step in sample processing is the centrifugation of the blood collection tubes. Specimens should be spun for ten minutes at a speed of 1100 to 1300 relative centrifugal force (RCF) in a swinging bucket centrifuge. A fifteen-minute spin at the same speed is required for spinning tubes in a fixed-angle centrifuge. Serum and plasma tubes without gel can be spun at a speed of 1000 RCF for ten minutes. It is important to spin gel tubes for the recommended time. The gel barrier in the tubes needs time to move and form a solid barrier between the red cells and the serum or plasma. Also, in PST tubes, the white blood cells and platelets that remain in the plasma need adequate time to spin out of the plasma. If the PST tubes are spun for less than the recommended 10 minutes, these cells and platelets may remain in the plasma and could cause interference with some chemistry analytes. It is recommended that SST tubes should not be re-centrifuged after their initial centrifugation. Re-spinning the tubes can result in elevated potassium values, as excess serum that has been in contact with the red cells will be expressed from underneath the gel barrier. Special Handling of Blood Specimens: Certain chemistry analytes will require the tube of blood to be chilled after collection in order to maintain the stability of the analyte. A slurry of ice and water is recommended for chilling the tubes of blood. Examples of specimens that need to be chilled or transported on ice include adrenocorticotrophic hormone (ACTH), angiotensin converting enzyme (ACE), acetone, ammonia, catecholamines, free fatty acids, lactate acid, pyruvate and renin. Other analytes are photo-sensitive, and need to be protected from light in order to remain stable and to ensure that the laboratory reports an accurate result. This can be done by wrapping the tube of blood in aluminum foil. The most common example of a light-sensitive analyte is bilirubin. Other chemistry analytes that need to be light-protected include beta-carotene and erythrocyte protoporphyrin. Stability for Whole Blood, Serum and Plasma: A whole blood specimen that is going to be spun down should be

Specimen Collection

- **Order of Draw:** Following the correct order of draw during venipuncture will help to ensure accurate test results.
- An example of improper order of draw that can lead to an incorrect chemistry result is drawing an EDTA tube prior to a SST or heparin tube for chemistry testing. The potential cross contamination of K₃ or K₂EDTA on the needle from the lavender top tube to the chemistry tube can lead to an elevated potassium result.
- **Proper Tube Mixing:** All tubes with additives need to be inverted to mix the additive evenly with the blood. Plastic serum tubes and SST tubes contain clot activator and should be inverted 5 times to mix the activator with the blood and help the specimen clot completely. Other additive tubes, such as heparin, need to be inverted 8-10 times to mix the anticoagulant with the blood and prevent clotting. Be sure that tubes are not being shaken vigorously, as this can lead to a hemolysis.
- **Correct Specimen Volume:** All blood collection tubes need to be filled to the correct volume. This will ensure the proper blood to additive ratio. For example, if a 5 mL draw heparin tube is only filled with 3 mL of blood, the heparin concentration is erroneously high and may potentially interfere with some chemistry analytes.
- Expiration dates should also be checked on the evacuated tubes. Expired tubes should not be used, as they may have a decreased vacuum, as well as potential changes in any additives in the tubes.