# Pre-Analytic Issues in Laboratory Medicine

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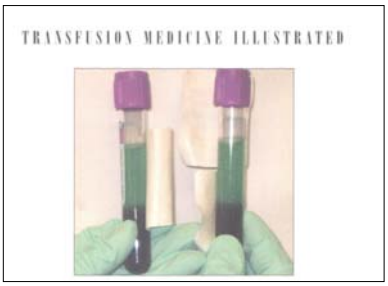
## Learning Objectives
## Pre-Analytic Issues in Laboratory Medicine

- What is the impact of posture on laboratory analytes?
- Be able to describe the common additives to blood specimens and when they are used
- Be able to describe the common causes of hemolysis and clot formation in the pre-analytic setting

## Pre-Analytic or Extra-Terrestrial?




## 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 are preanalytical handling (e.g. container, collection method, mixing) and biological variation.
- Analytical variability can also affect interpretation of a result

## Why are Pre-Analytical Issues Important?

- **Biological Variation** is due to factors inherent to the patient that may or may not be controllable
- **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, accurate results and interpretable results.

## 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

## Test Ordering

- Order the relevant test
- Know when to order
- Know how often to order
- Know how the results will be used
- If you don't know, consult with the laboratory director

## Patient Identification

- It is important to identify a patient properly so that a specimen is collected from the correct patient.
- .1% - 1% of specimens are from the wrong patient.

## 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.

## Proper Patient Identification Procedures

- CUMC policy requires two patient identifiers be used in all care settings.
- Hospital inpatients should be wearing an identification band. Blood should not be drawn from a patient without a identification band.
- Test forms should be compared to the inpatient's wrist band and to specimen labels or verbally confirmed with an outpatient.

## Avoiding Specimen Labeling Errors

- Draw and label specimens at the bedside, one patient at a time
- Affix specimen labels to the specimens immediately after collection
- Do not draw extra unlabeled tubes
- The person collecting 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
- Effect of Tourniquet Time
- Collection from IVs and Catheters
- Volume effects
- Avoiding Clots
- Avoiding Hemolysis

## 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 erect 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 edema.

## 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%.

## 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

## Collection Tube Additives

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

## Heparin (Anti-Coagulant)

- 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 (Anti-Coagulant)

- $K_2$EDTA 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 $K_2$EDTA from $K_3$EDTA for hematology to reduce affect on RBC parameters

## 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 EDTA, allowing the binding of pre-formed antibodies.
- Sodium citrate tubes are sometimes collected to obtain more accurate platelet counts.

## Citrate (Anti-Coagulant)

- Citrate is 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% of assigned collection volume

## NaF + K Oxalate (Anti- Glycolytic and Anti-Coagulant)

- 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 or patients 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

## Clot Activator (Shortens Time to Clot)

- In vitro activation of clotting system to enhance clot formation
- A silica clot activator is attached to the tube wall with a silicone surfactant
- Requires mixing for optimal function
- Time to clot is 15-30 minutes instead of 1 hour

## Serum Separator Gel (Separates Serum from Cells)

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

## Collection Tube Additives

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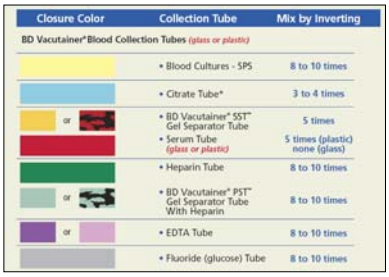
## Order of Draw




## Duration of Tourniquet Application (Metabolic and Concentrating Effect)

- 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 from IVs and Catheters (Dilutional Effect)

- 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 using a new sample drawn from a different site

## 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 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

## Syringe Collection (Hemolysis)

- Visual hemolysis was found in 19% of specimens drawn by syringe, compared to 3% when drawn by an evacuated tube system.
- The following reduce the incidence of hemolysis:
  - Pumping the plunger 2-3 times prior to collection to loosen the plunger.
  - Using a 3-10 mL syringe
  - Ensuring that the speed of aspiration does not exceed 1mL of air space during collection. Excessive aspiration forces cause hemolysis.
  - Filling the 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.
  - Using a larger bore needle
  - Not transferring specimen between containers

## 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

## 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

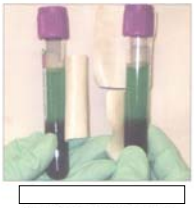
## Arterial Blood Gases

- Avoid air contamination from a bubble or uncapped specimen
- Delay in analysis can cause high $pO_2$ to fall or low $pO_2$ 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

## Iatrogenic Green Plasma due to Isosulfan Blue Dye



*Julie L. Houseworth, Judi A. Parks, Evangeline A. Miguel, Leo J. McCarthy, and Constance F.M. 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 emerald green color, and a newly collected specimen received about 0.5 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 ioxhexiritum 99. The dye was used to enable precise identification of sentinel nodes by afferent blue channels to facilitate a bilateral lymphadenectomy.

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## Discussion