Course Prerequisites:

- Venipuncture Modules 1, 2, 3, 4, 5 and 6

Course Goals and Objectives:

**Goal**

This course will cover circumstances associated with sample integrity.

**Course Objectives**

At the completion of this course, the learner will be able to:

- Identify complications associated with blood collection and possible effects on client safety and specimen integrity.
- Describe factors and circumstances that may result in harm to the client or reduce specimen integrity, and appropriate corrective measures.
- Describe the importance of standard precautions in prevention of harm to the client, the phlebotomist, and other healthcare workers.
7 SAMPLE INTEGRITY FROM COLLECTION TO TESTING

The accuracy of test results derived from blood samples can be greatly affected by the procedure used to collect the specimens and the manner in which they are handled following collection. This module introduces participants to the importance of proper collection and handling technique in maintaining specimen integrity.

Module 7 Objectives

At the end of this module, the learner will be able to:
1. Describe the importance of proper collection technique in maintaining the integrity of diagnostic blood specimens.
2. List the tests that cannot be performed on gel-barrier tubes.
3. Outline the effects of delay in transport and testing on the accuracy of test results.
4. Explain the effects of refrigeration, haemolysis and haemoconcentration on potassium levels.
5. Describe the test analytes that are affected by severe and moderate haemolysis.
6. Discuss the importance of ensuring complete fills of blood collection tubes.
7. Describe the affect of delay in testing on glucose results.
8. Describe measures to reduce glycolysis and stabilize glucose levels prior to testing.
9. Discuss the necessity of collecting a discard tube prior to collection of specimens for coagulation studies.
10. List the tests that require blood specimens to be transported on ice.
11. Explain the reason that tests require protection of blood specimens from light.
12. Tabulate the tests that require blood specimens to be held and transported at 37°C.
13. Explain, with reasons, that the laboratory may reject specimens.

7.1 Specimen Integrity

7.1.1 Blood collection equipment

Compatibility of blood collection system components is the responsibility of tube manufacturer. Inter mixing different manufacturers components may result in specimen problems such as haemolysis, needle/holder disengagement and short draws. The user must validate compatibility if mixing components from different manufacturers.

Manufacturer must ensure that fill accuracy is within 10% of stated draw throughout the tube shelf life. Solution strengths must be within 5% of that stated on the label throughout tube shelf life.

7.1.2 Specimen mixing

All tubes containing additives such as clot activators and anticoagulants must be gently inverted 8-10 times following collection, to ensure optimal additive activity.
7.1.3 Specimen volume

The proper amount of blood should be drawn into tubes to ensure adequate anticoagulation. Excess anticoagulant compared to the volume of blood can result in changes to cellular morphology (e.g. excess EDTA can shrink red cells and affect hematocrit, mean cell volume, etc.). Fill all anticoagulated tubes to completion – let the tube vacuum do its job!

7.1.4 Centrifugation

Specimens collected in anticoagulant tubes can be centrifuged minutes after collection; however, specimens collected in tubes without anticoagulant must be given time to clot completely prior to centrifugation and separation of serum from cells. Blood normally clots within 60 minutes at room temperature; although, it may take longer if the patient is on anticoagulant therapy. Chilling will also delay clotting.

If clotting time is insufficient, latent fibrin formation may occur presenting a problem for laboratory instruments. Adding a clot activator can accelerate clotting. The addition of thrombin will produce clot formation in approximately 5 minutes. Whereas, glass or silica particles additives, such as those found in SST evacuated tubes, allows clot formation in as little as 15 minutes.

It is not recommended to rim the tube to release clots attached to the tube stopper or the sides of the tube. Rimming the tube may result in haemolysis of the blood sample. The problem of clot “hanging” has almost entirely been removed by improvements in tube and stopper design.

7.1.5 Tube additives

EDTA salts (e.g. potassium EDTA) prevent blood from clotting through chelation or binding to calcium and other divalent ions. Although an excellent preservative for blood cells (haematology), EDTA salts should not be used for calcium, magnesium, iron, alkaline phosphatase, creatinine kinase, leucine aminopeptidase, and metal ion dependent enzymes.

The gel used in gel-barrier tubes may interfere with the following test analytes:

- Ionized calcium
- Progesterone
- Therapeutic drugs (including alcohol)
- Direct antiglobulin test (blood bank)
7.1.6 Haemolysis

Haemolysis can affect numerous test analytes, and variation in test results is related to the extent of haemolysis.

Seriously affected analytes include:
- Lactic Dehydrogenase (↑)
- AST (↑)
- Potassium (↑)
- Plasma haemoglobin (↑)

Noticeably affected analytes include:
- Iron (↑)
- ALT (↑)
- T4 (↓)

Slightly affected analytes include:
- Phosphorous (↑)
- Total protein (↑)
- Magnesium (↑)
- Calcium (↑)
- Acid phosphatase (↑)

7.2 Specific analyte interferences

7.2.1 Alcohol testing

Interference of blood ethanol analysis may occur due to use of alcohol swabs to cleanse the venipuncture site. Differing opinions exist on the effects of alcohol swabs on subsequent alcohol testing with some investigators reporting statistically significant increases in blood ethanol levels of 0.05 g/L (Peek et al. 1989), and some claiming that there is no effect.

The current CLSI standard for blood alcohol testing (Blood Alcohol Level Testing in the Clinical Laboratory; Approved Standard 1997) recommends against the use of alcohol pads to cleanse the venipuncture site.

7.2.2 Complete blood count

When the ratio of EDTA to blood is too high (incomplete tube fill), red cells shrink affecting hematocrit, mean cell volume and mean corpuscular haemoglobin concentration results. Erythrocyte sedimentation rates may also be adversely affected by excessive anticoagulant resulting from an incomplete tube fill (short-draw).
7.2.3 Drug level testing

Contact of blood with tube stoppers may result in contamination of blood samples for toxicology and therapeutic drug monitoring. Special tubes are available for trace elements that minimize tube stopper contamination.

The gel barrier may interfere with a number of tests including—Lactic dehydrogenase, ionized calcium, progesterone, tricyclic anti-depressants, and therapeutic drugs such as anti-epileptic, cardiac function, and aminoglycosides.

7.2.4 Potassium level testing

Potassium levels are 25 times higher inside cells than in extra cellular fluids (serum/plasma); therefore, haemolysed samples are unacceptable for testing, as potassium results will be falsely elevated. Even very small amounts of haemolysis can have a significant effect on the end result. Micro amounts of haemolysis may not be visible to the naked eye.

Haemoconcentration may also result in falsely elevated potassium levels from specimens collected in these areas. Haemoconcentration can result from pumping the fist, extended tourniquet use, edema, haematomas, and any other conditions that result in restricted blood flow.

Delay in testing (greater than 2 hours) and refrigeration can also alter potassium test results (see Specimen Storage below).

7.2.5 Glucose level testing

Serum/plasma glucose levels decrease approximately 5 mg/dL to 10 mg/dL per hour, and can be much higher if white blood cells are increased and/or specimen is contaminated with bacteria. Glucose breakdown (glycolysis) can be slowed by:

- Refrigerating specimens during storage and transport,
- Centrifugation of gel-barrier tubes as soon as possible after complete clot has formed
- Collecting specimens for glucose determination in tubes containing an antiglycolytic agent (e.g. sodium fluoride – grey-stopper evacuated tube).

Additives such as sodium fluoride prevent concentration changes in blood specimens over extended periods of time: the antiglycolytic action of sodium fluoride stabilizes glucose in the presence of red blood cells. Specimens can be reliably stored for up to 24 hours at room temperature and 48 hours at 2-8°C.

Glucose testing should be performed within one hour of collection for most accurate results if measures such as those described above to prevent glycolysis are not used.
7.2.6 Coagulation level testing

Discard tube

Drawing a discard tube prior to collection of a sodium citrate tube for PT/INR and APTT has been shown not to be of value. Numerous studies found no significant difference between PT/INR and APTT specimens collected with and without a discard tube. The CLSI does recommend collection of a discard tube when collecting specimens for special factor assays and when the facility policy establishes use of a discard tube for all coagulation tests when the citrate tube is the first or only tube drawn.

However, drawing a discard tube prior to collection of specimens for coagulation studies IS necessary when collecting with a winged infusion device due to the loss of tube vacuum that occurs as air is displaced in the tubing. Tubes for coagulation studies MUST be completely filled in order to ensure the required 1:9 ratio of anticoagulant to blood.
7.3 Specimen storage

Serum or plasma should be physically separated from the red blood cells as soon as possible. A maximum of 2 hours from the time of collection to separation of serum/plasma from cells by centrifugation is recommended. After 2 hours, certain test analytes will not be accurately represented in the specimen – the most susceptible analytes are potassium, glucose, and ionized calcium. Glucose levels are significantly decreased, while potassium is significantly increased due to cellular metabolism. By eight hours, tests such as iron are also significantly decreased. Some other analytes are more stable and are not affected as readily.

Although refrigeration helps inhibit or slow blood cell metabolism thereby stabilizing analytes that may be affected by changes in temperature, it is recommended that serum or plasma be removed from the red cells before samples are refrigerated. However, this is not always possible. General lab practice involves refrigeration of whole blood samples as well as separated serum and plasma samples until such time as they are further processed.

Potassium levels will be further falsely elevated in refrigerated specimens due to inhibition of the sodium-potassium-ATPase pump causing cells to leak potassium into the plasma or serum that will be used for testing. Cells contain 25 times the level of potassium as serum/plasma. Refrigeration can lead to falsely elevated potassium levels in serum/plasma in a relatively short time period.

PT/INR test results are stable for up to 24 hours at room temperature prior to removal of the tube stopper. Specimens for PT/INR testing should not be refrigerated as results may be altered by cold activation of Factor VII.

Activated partial thromboplastin times (APTTs) are stable for 4 hours at room or refrigerated temperatures, and complete blood counts are stable for 24 hours at room temperature. Tubes should be kept closed at all times. Some tests are affected by changes in pH when the sample is exposed to air: loss of carbon dioxide causes increased pH levels, and decreases in analytes such as ionized calcium and acid phosphatase. Keeping the tubes closed also reduces contamination, evaporation, and the possibility of spills and aerosols. If serum separators must be added to blood tubes prior to centrifugation, the stopper should be carefully removed to avoid aerosol production, and the tubes covered before centrifugation.

Elevated temperatures affect the stability of the blood sample: samples should never be stored or transported at temperatures above 35°C. Rapid deterioration of the sample will occur at elevated temperatures causing erroneous results.

Likewise, greatly reduced temperatures can affect sample stability. Whole blood should never be stored or transported at temperatures below zero degrees, as freezing will cause haemolysis.
Some samples must be chilled at 2-8 °C immediately after collection. Samples can be chilled by placing in crushed ice or a mixture of ice and water to a level above the contents of the tube. Large ice cubes should not be used because they do not provide adequate contact with the blood sample.

**Chilling is recommended for:**

- Catecholamines,
- pH/blood gases,
- Ammonia,
- Lactic acid
- Pyruvates,
- Gastrin
- Parathyroid hormone (PTH)

Specimens for potassium testing should not be chilled for more than 2 hours, as glycolysis, which provides energy for pumping potassium into cells, is inhibited by cold temperatures. Specimens for tests such as ACTH (adrenocorticotropic hormone) and cortisol determinations should also not be chilled for longer than 2 hours.

### 7.4 Specimen transport

#### 7.4.1 On-site transport of blood specimens

Specimens should be transported in as short a time as possible. Tubes should be transported and stored in a vertical position, stoppers up to promote clot formation and reduce agitation of the specimen and the potential for haemolysis. The stopper is also less likely to come off in this position.

Blood specimens should be handled gently to avoid red blood cell damage and haemolysis. Tests seriously affected by moderate (1%) haemolysis include LD, AST, K, and plasma haemoglobin, which are all falsely elevated; and T4, which is falsely decreased. Tests slightly affected by moderate haemolysis include phosphorous, total protein, albumin, magnesium, calcium, and acid phosphatase, which are all falsely elevated.

Exposure to light will adversely affect tests for analytes sensitive to light. These include vitamin A and B₆, beta-carotene, porphyrins, and bilirubin. Specimens for any of these tests should be wrapped in aluminum foil immediately after collection for transport to the laboratory.

Pneumatic tubes used in some institutions to provide rapid transport of specimens may affect tests most affected by red cell disruption and haemolysis. This includes LD, potassium, plasma haemoglobin, and acid phosphatase. Transport by pneumatic tube system does not affect the following tests: albumin, sodium, total bilirubin, total protein, urea nitrogen, uric acid, leukocyte count, PTT, and PT.
7.4.2 Off-site transport of blood specimens

If transport to the laboratory will take longer than 2 hours, blood samples should be centrifuged and the serum or plasma physically separated from the cells. This can be accomplished with gel barrier tubes or by transferring serum or plasma to another tube after centrifuging. Pay particular attention to proper labelling of transfer tubes.

 Serum can remain in contact with the gel barrier for at least 24 hours if refrigerated and kept in a vertical position with the stopper up. Separated serum or plasma may be held up to 8 hours at room temperature, or refrigerated. If the time from collection until testing is to exceed 48 hours, the serum or plasma should be frozen at -20C. Serum and plasma should not be thawed and refrozen: it should be frozen only once.

 Temperatures that are too hot (summer) and too cold (winter) must be avoided. If specimens must be transported in these conditions they should be placed in an insulated container to protect them from temperature changes. If necessary, cool or warm packs may be required at different times of the year in our Canadian climate.

 Specimens for lead, zinc, protoporphyrin, and cyclosporin determinations should not be centrifuged. These tests are performed on unseparated anticoagulated whole blood.

 Specimens should be packaged to prevent damage and contamination due to spill, leakage, or breakage. The contents of the specimen container should not be released into the environment on transport. Combination packaging should be used consisting of a watertight inner packaging into which the specimen container is placed: an absorbent material is placed between the two packaging layers, and the accompanying requisition is placed outside the watertight package. This will prevent spill or leakage of specimen contents if the primary container should break in transit. The specimen is then placed into a secondary container of adequate strength for its capacity and intended use.

 Specimens that must be transported on ice (chilled):

- Ammonia
- Lactic acid
- Pyruvate
- Gastrin
- ACTH
- Some coagulation studies
- Parathyroid hormone (depending on requirements of institution)
Specimens sensitive to light:

- Bilirubin
- Beta-carotene
- Vitamins A, B₆
- Porphyrins

Specimens that must be held and transported at 37°C

- Cryoglobulins
- Cold agglutinins

7.5 Specimen rejection criteria

There are a number of reasons to reject specimens that are received for laboratory testing. Specimens must be received in a condition that will not affect the accuracy of test results derived from them. Following are some of the common reasons for specimen rejection:

- **Inadequate or improper identification**
  Specimens should not be processed if they are received unlabelled or are mislabelled (name and identification on specimen label does not match that on the accompanying requisition).

- **Inadequate volume of specimen**
  The proper ratio of tube additive to blood must be achieved. Test results will be adversely affected if less blood is drawn than required.

  **Examples:**
  
<table>
<thead>
<tr>
<th>Sodium citrate:</th>
<th>Prothrombin time</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA:</td>
<td>Cell count, cell morphology, lipids</td>
</tr>
<tr>
<td>Heparin:</td>
<td>Creatine kinase (CK), aminoglycosides</td>
</tr>
</tbody>
</table>

- **Using the wrong collection tube**
  Additives can interfere with test results if not specified for that test.

  **Examples:**
  Sodium fluoride: Urease urea nitrogen method
• **Wrong order of draw**

Wrong order of draw during multiple blood specimen collection may result in contamination of non-additive tubes with anticoagulant. It is unlikely that the laboratory will recognize that the wrong order of draw was followed; therefore, it is the responsibility of the phlebotomist to ensure that proper order of draw is followed to ensure accuracy of patient test results.

• **Haemolysis**

Haemolysis may adversely affect some test results; therefore, even moderately haemolysed specimens should be rejected and not be processed.

• **Improper transport**

Specimens should be rejected when:

- specimens received in the laboratory un-chilled when handling instructions specified chilling
- specimens that have been exposed to light when the analyte being tested for is light sensitive
- serum or plasma samples received unfrozen when they should have been frozen
- excessive delay in delivering specimens to the laboratory

- **Clotted blood in anticoagulant tube**
- **Specimens without a requisition**
- **Leaking or obviously contaminated containers**
- **Specimens received without proper specimen containers**
References:


