Department of Pathology and Laboratory Medicine

SPECIMEN COLLECTION, HANDLING, and TRANSPORT

Purpose
Laboratory testing provides information about a patient’s health to assist Physicians in diagnostic and therapeutic decisions. Specimen Integrity is dependent on accurate pre-analytical processes to include patient preparation, specimen collection, handling, and transportation. Improper collection and handling of samples can give erroneous results and compromise the care of the patient. These guidelines cover some of the key steps in handling blood samples to provide optimal specimens for testing.

Principle
There are multiple Pre-Analytical factors associated with the handling and processing of laboratory specimens that can lead to test result inaccuracy. Strict adherence to all phases of proper collection and processing is essential for accurate test results.

Pre-Analytical Factors:
1. Specimen Collection:
   a. Improper Patient Identification
   b. Incorrect Order of Draw
   c. Incorrect Tube Selection
   d. Traumatic draws leading to hemolysis
   e. Inadequate mixing or insufficient sample

2. Specimen Handling/Processing:
   a. Serum tubes not thoroughly clotted before centrifugation
   b. Delay in Centrifugation
   c. Storing specimens in incorrect temperatures

3. Specimen Transportation
   a. Frozen specimens thawing during transport
   b. Unspun specimens transported >2 hours from collection

Specimen Collection
Patient results are only as good as the specimen collected. The integrity of the sample must be preserved and requirements for collection and handling must be followed. It is critical that adequate volumes are collected on each patient and the patient preparation is adhered to follow test requirements such as fasting.

1. Collection Process: Please refer to the Venipuncture Procedure listed on the Laboratory Test Menu found on www.Lexmed.com or LMC Intranet Lexloop. Following proper phlebotomy techniques will assist in preventing inaccurate test results:
   a. Tourniquet left on <1 minute to prevent Hemolysis
   b. All tubes collected must be collected in the correct Order of Draw and inverted gently to ensure proper mixing of additive or anticoagulant:
<table>
<thead>
<tr>
<th>Closure Color</th>
<th>Collection Tube</th>
<th>Mix by Inverting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>Blood Cultures - SPS</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>Yellow or Red</td>
<td>Citrate Tube*</td>
<td>3 to 4 times</td>
</tr>
<tr>
<td>Yellow</td>
<td>BD Vacutainer® SST Gel Separator Tube</td>
<td>5 times</td>
</tr>
<tr>
<td>Yellow or Red</td>
<td>Serum Tube (glass or plastic)</td>
<td>5 times (plastic) none (glass)</td>
</tr>
<tr>
<td>Orange</td>
<td>BD Vacutainer® Rapid Serum Tube (RST)</td>
<td>5 to 6 times</td>
</tr>
<tr>
<td>Green</td>
<td>BD Vacutainer® PST Gel Separator Tube With Heparin</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>Purple or Pink</td>
<td>Heparin Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>Purple</td>
<td>EDTA Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>Purple</td>
<td>BD Vacutainer® PPT Separator Tube K&lt;sub&gt;2&lt;/sub&gt;EDTA with Gel</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>Purple</td>
<td>Fluoride (glucose) Tube</td>
<td>8 to 10 times</td>
</tr>
</tbody>
</table>

Note: Gold top tubes (SST) contains an additive (Clot activator) and must be inverted.
c. All collection tubes must be filled with the required volume (no short samples). Fill lines are indicated by the black and white notches on the side of the label:

d. Do not use expired tubes. Expiration dates can be found on each paper label on the tube

e. Incorrect Order of Draw will introduce contamination with anticoagulants and often produce inaccurate results. An example would be increased Potassium if the Lavender tube is drawn prior to collection of Gold.

2. Specimen Labeling: All specimens must contain two specimen identifiers:
   a. Patient’s first and last name
   b. Patient’s Date of Birth
   c. Unique Identifying number to assist with identification such as Social Security number

   Note: Hospital policy requires Patient’s first and last name and Date of birth.

Specimen Handling and Processing (Centrifugation)

1. Serum tubes must be placed in an upright vertical position and allowed to clot for a minimum of 30 minutes before centrifuging. After the specimen has been allowed to fully clot, the tube is to be centrifuged within 1 hour of collection and no longer than 2 hours after collection.

   ** Failure to separate red cells from serum or plasma within 2 hours of collection, may lead to inaccurate results**

   Note: Patients on anticoagulant therapy may need longer time to clot.

2. Centrifugation: All serum tubes must be properly balanced and tubes spun within the appropriate speed and time based on the centrifuge in use. The centrifuges listed below are the only models approved for separation of blood samples.
<table>
<thead>
<tr>
<th>Centrifuge Name</th>
<th>Type</th>
<th>RPM</th>
<th>Time</th>
<th>Directions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon Mini 642B</td>
<td>Horizontal</td>
<td>Manufacturer Set (3380 RPM –1590g)</td>
<td>Manually Set Time to 10</td>
<td>Close lid and turn dial to 10</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td>minutes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizon Mini 642E</td>
<td>Horizontal</td>
<td>Manufacturer Set (3380 RPM –1590g)</td>
<td>Manufacture set to 10</td>
<td>Close lid and Press Start</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizon 653V</td>
<td>Horizontal</td>
<td>3200 RPM - 1490g</td>
<td>Manufacture set to 10</td>
<td>Turn dial to 3.2 RPM, Close Lad</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td>and Press Start</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizon 653ES</td>
<td>Horizontal</td>
<td>Manufacturer Set 3150 RPM – 1550g</td>
<td>Manually set time to 10</td>
<td>Select 10 minutes, Close lid and</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td>Press Start</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizon 642VFD-PLUS</td>
<td>Horizontal</td>
<td>Manufacturer Set 3800 RPM – 2000g</td>
<td>Manufacture set to 10</td>
<td>Close lid and Press Start</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizon 755VES</td>
<td>Horizontal</td>
<td>Manually set to 3500 RPM</td>
<td>Manually set time to 10</td>
<td>Select RPM to 3500 and Select</td>
</tr>
<tr>
<td>(Drucker)</td>
<td>Rotor</td>
<td></td>
<td>minutes.</td>
<td>time to 10 Minutes. Press Start</td>
</tr>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

3. Observe each tube after centrifugation. Verify that the gel is completely separating cells from serum. If complete separation is not visible, **DO NOT RECENTRIFUGE**.

a. Transfer serum or plasma to an aliquot tube using a pipette leaving a small amount on top of the gel or packed cells.

b. Centrifuge the aliquot tube following the recommendations of your centrifuge.

c. Observe the aliquot tube for red cells at the bottom of the tube (red button). Transfer the serum/plasma into another aliquot tube leaving a layer of serum/plasma above the red button.

**Limitations:**

*Do not disturb the red button which are the red cells that have been separated.*

- Label each aliquot tube to properly identify your patient through each step of processing.
- Do not place unspun tubes in the refrigerator. The refrigeration of the unspun tube will increase Potassium levels by 135%
- Light Sensitive requirement: Pour plasma/serum into a dark aliquot tube to protect the specimen from any light source to ensure specimen integrity. If a dark aliquot tube is not available, wrap aluminum foil or paper towel around the tube (not the stopper) tightly. Please ensure the tube will not be exposed to light during storage and transport. The patient identification should be easily accessible to view by sliding the tube from the wrapping which is why the stopper end of the tube is not covered.

**Specimen Transport**

To minimize exposure to bloodborne pathogens in transport of specimens, Standard Precautions must be used. **ALL** blood and other potentially infectious material are treated as if they are known to be infectious with HIV or hepatitis and other bloodborne pathogens. All specimens must be transported in a sealed biohazard bag. Please refer to the Laboratory Test Directory on [www.lexmed.com](http://www.lexmed.com) or LMC Intranet – Lexloop for specific storage requirements (room temp (ambient), refrigeration, or frozen) for the testing of the patient sample.
1. **Room Temp Requirements:** If your specimen does not have a specific storage requirement and will be stored at room temp before Courier pickup, please place in a sealed orange/red biohazard labeled specimen bag.

   *Note: Do not store tubes in direct contact of a heat source such as direct sunlight, top of refrigerator, heating/air vents, etc.*

2. **Refrigeration:** If your specimen requires refrigerated temperatures during transport, place in a “blue” biohazard labeled specimen bag (see picture below under #3). Please use a permanent marker and place an “X” in the box designating “Refrigerator” temperature. Place the specimen in your refrigerator until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on ice to retain the refrigerator temperature.

   *Note: Never store unspun serum/plasma tubes in refrigerator. Tubes must be centrifuged before storage to ensure specimen integrity.*

3. **Frozen:** If your test requires the specimen to be frozen after processing, the specimen must be centrifuged and serum/plasma must be transferred to an aliquot tube by pipette without disturbing gel or packed cells. Following labeling requirements for all aliquots. The aliquot is to be placed in a blue biohazard labeled specimen bag. Please use a permanent marker and place an “X” in the box designating “Frozen” temperature. Place specimen bag in your freezer (or on dry ice) until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on dry ice to retain the frozen temperature.

   *Note: Never place original collected tube in freezer. Freeze only the labeled aliquot sample.*

4. **STAT:** If your specimen has a “STAT” priority, please call your Courier for pickup. Place the sample in a “Red” biohazard labeled specimen bag. The red bag will easily be seen as a STAT specimen upon delivery to the laboratory.

5. **Other Requirements:**
   - Remove all needles and sharps from all specimens before transporting.
● All specimens transported via courier must be transported in sealed biohazard, leak-proof, puncture resistant container tightly closed before transportation.

● Please place specimens in the Ziploc portion of the specimen bag. Completed requisition is to be placed in the outside pocket.

REFERENCES

1. Becton Dickinson (BD) Diagnostics Preanalytical Systems educational aids:
   ● Mix tubes by Inverting the Recommended Number of Times, 2010.
   ● How to Prepare a Quality Sample Using BD Vacutainer SST Tubes, 2010.
   ● Troubleshooting Hemolysis Issues, 2010.

2. Drucker Diagnostics.com - Horizon Centrifuge Owner’s Manuals:
   ● Horizon Model 642B Laboratory Centrifuge Owner’s Manual P/N 03-0-0002-0078 Rev. C
   ● Horizon Model 642E Laboratory Centrifuge Owner’s Manual P/N 03-0-0002-0039 Rev. G
   ● Horizon Model 653V Laboratory Centrifuge Owner’s Manual P/N 7711015 Rev. B
   ● Horizon Model 653ES Laboratory Centrifuge Owner’s Manual P/N 7711014 Rev. B
   ● Horizon Model 642VFD Laboratory Centrifuge Owner’s Manual P/N 7711046 Rev. C
   ● Horizon Model 755VES Laboratory Centrifuge Owner’s Manual P/N 7711010 Rev. B