SAMPLE COLLECTION, TRANSPORTATION & STORAGE

નિસ્ત્રક નિદાન તાલીમ પ્રો ગ્રામ
Objectives

- Basic Concept Of Blood Collection
- Specimen Containers
- Sample Rejection Criteria
- Specimen Transport
- Specimen Storage
- Sample Processing
Types of Biospecimens: Blood

- Plasma – Liquid portion of the Blood without cells
- Serum – Liquid portion of the Blood without cells and fibrinogen
- Lyophilized – Dehydrated Frozen sample containing only constituents
- Lymphocytes
- Erythrocytes
- Platelets
Materials Required To Collect The Blood Sample

- Sterilized Blood Collecting Devices
- Needles And Syringes
- Vacuum Based Tubes
- Lancets For Finger Prick
- Spirit/70% Alcohol
- Tourniquet For Venipuncture/Phlebotomy

Materials To Follow Standard Work Precautions

- Gloves
- Puncture Resistant Discards Jar Containing Freshly Made 1% Sod. Hypochlorite Solution
- Adsorbent Material Sheet With Plastic On One Side To Prevent Blood Seepage Onto The Working Bench
- Needle Destroyer
Specimen Collection
Venipuncture: From The Vein
Syringe Draw: From The Vein
Dermal Puncture: From The Finger or Heel
   -(In Infants – 6 Weeks To 4 Months)
Labeling Of Specimen
Name of The Laboratory, Name of The Client, Identifying Number, Date/Time of Collection, Name of Investigations
Procedure For Blood Collection

1. Introduce yourself & identify the Patient
   (It is the most Important Specimen Collection Point)

2. Collect Required Items (Needles, Tubes, etc.)

3. Label vials & verify the same with Patient Identification

4. Wash Hands & Wear Gloves

5. Prepare the Patient & Explain Procedure of Blood Collection
Procedure for drawing blood

Apply Tourniquet
(Do not leave it on for more than 1 minute)

Choose a Vein

Clean the Selected Vein, pierce it & take blood to a full draw (3-5 ml).

Obtain the required amount of blood (3-5 ml)

Remove Needle from the vein, apply pressure and simultaneously release the tourniquet
Procedure for drawing blood

10. If manual method (using needle/syringe) is being used, then pour the Blood in proper Vial compatible to the Test.

11. Destroy it using Needle Destroyer & Discard the Needle (in appropriate container)

12. Invert (mix) the whole blood tubes that contain anticoagulant 6-8 times to avoid clotting/do not invert, allow blood to clot if serum is required for testing

13.
Sterile Blood Needles; Sterile Syringes; Plain Vacutainer; Blood Tubes; Alcohol Prep Pads; Tourniquet
Blood Collection
<table>
<thead>
<tr>
<th>4. COLOUR OF VACUTAINER TUBES</th>
<th>ANTICOAGULANT</th>
<th>CHOICE OF SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red</strong></td>
<td>No Additive</td>
<td>Serum</td>
</tr>
<tr>
<td><strong>Green</strong></td>
<td>Sodium Hepain</td>
<td>WB or Plasma</td>
</tr>
<tr>
<td></td>
<td>Lithium Heparin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ammonium Heparin</td>
<td></td>
</tr>
<tr>
<td><strong>Lavender</strong></td>
<td>K$_2$EDTA or K$_3$EDTA</td>
<td>WB or Plasma</td>
</tr>
<tr>
<td><strong>Gray</strong></td>
<td>Sodium Fluoride/Potassium Oxalate</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>Sodium Fluoride/Na$_2$EDTA</td>
<td></td>
</tr>
<tr>
<td><strong>Light Blue</strong></td>
<td>Sodium Citrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTAD</td>
<td></td>
</tr>
</tbody>
</table>
Certain Factors Affect The Results of The Test (Collection Variables)

1. Diurnal Variation
2. Posture
3. Stasis
4. Haemolysis
5. Preservation
6. Patient Stress
7. Phlebotomy - Tube Draw Order
8. Heamodilution
1. **Diurnal Variation**: Some analytes show significant diurnal variations, so the time of collection for this samples should be ideally after an overnight fast.

- Analytes showing diurnal variation are: **Cortisol, Iron, Catecholamines, Glucose, Triglycerides**.

Within a day variation for these substances may as much as 30%-50%.
2. **Posture**: Posture of the patient at the time of collection can have a significant effect on protein and protein bound substances in the serum.

- e.g. **Total protein, Albumin, Lipids, Iron, Calcium, Enzymes**.

- When the patient goes from supine to the standing position, these serum constituents increase their concentration by 5%-15%.

- This effect is due to the movement of water out of the intravascular compartment on standing.
• 3. **Stasis**: Prolonged use of a tourniquet may elevate a no. of lab. results.

• Application of tourniquet for a long period, results in:
  • Stasis of blood → which increases blood pH, decrease PO2, increase PCO2 and increase the lactate concentration because lactate is produced by anaerobic metabolism.

• **Tourniquets should be avoided during the collection of samples for Blood gas analysis.**
4. **Hemolysis:** During sample collection and until the serum/plasma is isolated from the red cells care taken to minimise haemolysis.

Hemolysis may arise due to –

- Use of too large or too small a needle during collection.
- Moisture in the syringe.
- Vigorous mixing of blood in the tube.
- Emptying the syringe without removing the needle.
• Heamolysis leads to increase, falsely, the serum conc. of analytes present in high conc. within the RBCs.

• On the other hand, for those substances that exist at lower conc. in the red cells than outside, hemolysis will result in a dilution effect on the serum constituents.

• Common analytes whose conc. is significantly affected by hemolysis are Total Protein, Albumin, Lipids, Iron, Calcium, Potassium, Magnesium, Aldosterone, Enzymes, Bilirubin, Cholesterol, Renin, Nor epinephrine.
5. Preservation

For certain measurements, such as blood gas analysis & lactate estimations, are affected by RBC Glycolytic activity.

Samples drawn for these analytes must be placed immediately on ice bags and send to the laboratory as soon as possible within a period of 45 mins.
6. **Patient stress:**

- The stress of collection may also affect laboratory results.
- Anxiety of the patient may result in changes in **catecholamine** levels and also **Blood Gas** results through direct hormonal effects and hyperventilation.
- Every effort is made to calm the patient before collecting the sample.
7. Haemodilution:

- If blood must be drawn from a patient who has intravenous equipment attached to one arm, the blood sample should be drawn from a vein in the other area. If neither arm is free, an ankle vein is the site of choice for the venipuncture.
8. Phlebotomy = Tube Draw Order:

- Often it is necessary to draw blood into a number of different tubes for the same patient at the same draw time.
- It is important to prioritize phlebotomy tubes, so that material from one tube does not contaminate the next one.
- E.g. if an EDTA tube (lavender cap) is used before a regular serum tube (red cap) the latter tube becomes contaminated with trace amounts of EDTA which may inhibit certain serum enzyme estimations such as alkaline phosphatase.

The accepted draw order is to fill non-coagulated tube first and anticoagulated tubes next.
• **Frozen Specimens:** Serum or plasma specimens need to be frozen only if specifically stated in the specimen requirement.

• In these cases, it is essential to freeze the specimen as soon as it is separated from the cells. Always freeze specimens in plastic tubes unless specifically instructed otherwise. Glass tubes are not acceptable.

• Lay the tube in the freezer at a 45° angle to avoid tube breakage caused by expansion during freezing.
Transportation

- The time between sample collection and receipt by the lab should not exceed 45 min.
- During transportation, tubes should be kept in test-tube stand and in the stopper-up position to promote clot formation and reduce hemolysis and contamination with substances released from the stoppers.
Changes occurring in the Blood on keeping for a long time

1. CO2 conc. more in plasma so it diffuses $\rightarrow$ atmosphere. From the cells $\rightarrow$ diffuses in plasma $\rightarrow$ Causing increase in blood pH (alkaline)

pH change compensated by conversion of HCO3 $+(\text{H}^+)$ $\rightarrow$ to CO2 and water.

H+ ions obtained from other blood buffers and these changes occur in the cells $\rightarrow$ adequate amount of buffers and carbonic anhydrase.

Decrease in intracellular HCO3 ion $\rightarrow$ so diffusion occurs from plasma to the red cells. Where as chloride moves in the reverse direction to maintain electrical neutrality.
• 2 conversion of glucose to lactic acid by \textit{glycolysis}.

• 3 \textbf{Increase in plasma inorganic phosphate} due to hydrolysis of organic phosphate present in the red blood cells. To avoid this serum or plasma should be separated shortly after collection.
• 4  **Formation of ammonia** from nitrogenous substances, of which urea is the chief, may occur quickly. It is increased if the blood has been contaminated with bacteria.

• Urea----------→ Ammonia
• 5. **Passage of substances through the red cell membrane.**

• **Potassium and Phosphate** for example are present in much higher conc. In the cells than in the plasma.

• Serum or Heparinized Plasma should be separated shortly after collection.

• Diffusion of **Potassium** occurs more rapidly in blood at 4 degrees centigrade than at room temp., as the Na-pump is less active. Separated samples show no further changes in potassium or phosphate.
• To prevent such changes special handling such as:

• collection into ice chilled tubes \(\rightarrow\) and immediate centrifugation \(\rightarrow\) perform the test as soon as possible or freezing of the plasma/serum may be required at 4 to -20 degrees centigrade for up to 24 hours or frozen for longer periods.
Laboratory Criteria For Unacceptable Samples

1. **Inadequate Sample Identification:**
   
   - Minimum amount of patient information that must be included on each laboratory slip & specimen is: Name, Address, Indoor/outdoor no., Age, sex.
   - The Lab. Tech. should visually and verbally verify the identity of the patient, comparing the name & no. on test requisition form & label on the test tube.
   - Difference between the name on the lab. slip & the name on the sample container are the grounds for sample rejection.
2. **Inadequate Volume of Blood:**

A specific amount of additive is added to a phlebotomy tube, based on the presumption that the tube will be completely filled with blood.

For e.g. Preheparinising a syringe & then collecting an insufficient volume of blood may cause erroneous results.
3. **Improper Collection Tube:**

In general, *serum* is the preferred sample for most **Biochemical analysis**.

Sodium fluoride tubes designed for glucose sample are unsuitable.

Chelating agents are unacceptable for many enzymatic methods.

Heparin is the anticoagulant least likely to affect clinical chemistry procedures.
4. **Improper Transportation:**

Samples for blood gases, lactic acid, ammonia & other procedures where there is a significant sample lability must be transported to the laboratory on ice bags & delivered with in a specific time.
5. **Interferents**:  
The presence of potential analytical interferents such as Icteric Serum, Lipoaemia, Turbidity, Drugs, Dietary Components that may interfere with a specific analytical procedure is a basis for rejecting a sample.
<table>
<thead>
<tr>
<th>Color</th>
<th>Bio waste materials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yellow</strong></td>
<td>Blood sample, Urine sample, other Body fluid samples</td>
</tr>
<tr>
<td><strong>Red</strong></td>
<td>Plastic waste materials, I.V. line, Uro-bag, Drainage tube, catheter, gloves</td>
</tr>
<tr>
<td><strong>Blue</strong></td>
<td>Needle after cutting, glass syringe, scalpel, glass blood container</td>
</tr>
<tr>
<td><strong>Black</strong></td>
<td>Paper waste, food material and other laboratory waste</td>
</tr>
</tbody>
</table>
THANK YOU