Hemolysis and Pre-analytical Variables

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DESCRIPTION:
Hemolysis results when red blood cells are damaged or destroyed releasing hemoglobin. Hemolyzed specimens can result from patient conditions but most often result from procedural errors in specimen collection and handling. Numerous factors are associated with pre-analytical errors. These errors can compromise specimen integrity and impact patient care.

OBJECTIVES:
At the end of the session, participants will be able to:
- Recognize the cause of hemolysis.
- Discuss how to prevent hemolysis
- Determine how to reduce pre-analytical variables and specimen rejection
Hemolysis and Pre-Analytical Variables

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Objectives

- Recognize the cause of hemolysis
- Discuss how to prevent hemolysis
- Determine how to reduce pre-analytical variables and specimen rejection
What is Hemolysis?

- The breakage of the red blood cell's membrane
- Causes the release of hemoglobin and other internal components into the fluid
- Visually detected by a pink to red color in the serum or plasma
- A common occurrence
- Compromises laboratory testing

Hemolysis

- Can occur from two sources:
  - In-vivo usually do to pathologic conditions
    - Autoimmune Hemolytic Anemia
    - Transfusion Reaction
    - Toxins and Poisons
  - In-vitro
    - Improper collection
    - Specimen processing
    - Specimen transport
Degree of Hemolysis

Causes of Hemolysis

- Specimen Collection
- Prolonged tourniquet application
- Vein size and trauma
- Needle size
- Under filled tubes
- Slow flow of blood into collection tubes
- Alcohol preparation
- Syringe collection and transfer
- Cather Collection
- Milking a skin puncture
Causes of Hemolysis

- Specimen Processing
  - Vigorous mixing or shaking of the tubes
  - Not allowing the serum tube to clot properly
  - Use of applicator sticks to dislodge fibrin
  - Prolonged contact of serum or plasma with cells
  - Exposure of excessive heat or cold
  - Don’t over centrifuge or centrifuge at high speeds

Causes of Hemolysis

- Specimen Handling
  - Mechanical trauma during transport of a pneumatic tube system
  - Do not subject the specimen to significant jarring
  - Protect the specimen for transport
  - Don’t expose the specimen to extreme temperatures
Effect of Hemolysis

- Increase of certain analytes
- Interference in the test method
- Degree of hemolysis varies with the interference of laboratory results
- Quality Specimens = Quality Test Results

Hemolysis

- Destruction of red blood cells with the release of hemoglobin
- Analytes affected:
  - Potassium
  - Phosphorus
  - ALT
  - Coagulation
  - CK
  - Troponin T
  - Magnesium
  - Magnesium
  - Magnesium
  - Bilirubin
  - LD
  - Ammonia
  - Iron
  - Cholesterol
  - Sodium
  - Haptoglobin
  - Calcium
  - Sodiu
  - Calcium
  - Amylase
Effected Analytes

**Increase**
- Potassium
- Magnesium
- Iron
- LD
- Phosphorus
- Ammonia
- Total Protein
- Calcium

**Decrease**
- RBC count
- Hemoglobin
- Hematocrit
- Coagulation Factors
- Haptoglobin
- Troponin T

Laboratory Tests Affected by Hemolysis

<table>
<thead>
<tr>
<th>Seriously Affected</th>
<th>Noticeably Affected</th>
<th>Slightly Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (K)</td>
<td>Iron</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Lactic Dehydrogenase (LD)</td>
<td>Alanine Aminotransferase (ALT)</td>
<td>Alkaline Phosphatase (ALP)</td>
</tr>
<tr>
<td>CBC</td>
<td>Thyroxine (T4)</td>
<td>Total Protein (TP)</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>Albumin</td>
</tr>
<tr>
<td></td>
<td>APTT</td>
<td>Magnesium (Mg)</td>
</tr>
<tr>
<td></td>
<td>C-peptide</td>
<td>Calcium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rapid Plasma Reagin (RPR)</td>
</tr>
</tbody>
</table>
Hemoconcentration

- Intravascular pressure build up, allows analytes to escape through the capillary walls and into the tissues
- This results with an concentration of analytes in the circulatory system
- Biochemical changes take place in the trapped blood

Hemoconcentration
Possible Cause and Effects

- Leaving the tourniquet on the patient arm longer than two minutes.
- Asking or allowing the patient to pump their fist.
  - Changes in CBC results.
  - Changes in results of blood chemistries including Iron, Potassium, Magnesium, Calcium, Enzymes and Troponin T tests.
Prolonged Tourniquet Application

- Potassium
- Magnesium
- Ionic Calcium
- Albumin/Protein
- Decrease in pH
- WBC and Hemoglobin
- Cholesterol and Triglycerides
- Coagulation Factors
- Iron
- Ammonia

Hemoconcentration And Hemolysis

Hemoconcentration can lead to Hemolysis
Ways to Prevent Hemoconcentration

- Ask the patient to release the fist when blood appears in the first tube
- Choose the appropriate vein
- Do not allow the patient to continue pumping the fist
- Do not massage the area
- Do not slap the area
- Do not probe or redirect the needle in search of a vein
- Release the tourniquet within 1 minute

Rejected Specimens

- Preanalytical phase highly susceptible to error

- Hemolyzed specimens account for 60% of rejected specimens
Testing Errors

Preanalytical Variables

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Specimen Rejection

- Identity discrepancies
- Inadequate volume of blood
- Hemolyzed specimens
- Incorrect tubes
- Specimens improperly transported
- Anticoagulated specimens that contain clots
- Contaminated Specimens
- Timed sample drawn at the incorrect time

Importance of Preventing Hemolysis

- Impacts laboratory tests
- Higher rate of rejected specimens
- Usually requires repeat collection
- Delayed diagnosis
- Delayed treatment
- Additional discomfort for the patient
- Additional cost
- Frustration for the laboratory
Phlebotomy Practices

- Ordering
- Patient Identification
- Anticoagulant
- Tourniquet
- Traumatic Phlebotomy
- Tube Volume
- Tube Inversion

## Pre-Analytical

<table>
<thead>
<tr>
<th>Ordering</th>
<th>Collection</th>
<th>Collection</th>
<th>Processing</th>
<th>Transporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Additives</td>
<td>Specimen Volume</td>
<td>Separation</td>
<td>Light</td>
</tr>
<tr>
<td>Identification</td>
<td>Vein Selection</td>
<td>Short Draw</td>
<td>Centrifugation</td>
<td>Evaporation</td>
</tr>
<tr>
<td>Labeling</td>
<td>Order of Draw</td>
<td>Inverting</td>
<td>Hemolysis</td>
<td>Temperature</td>
</tr>
<tr>
<td>Diet</td>
<td>Cleansing</td>
<td>IV Lines and Line Draws</td>
<td>Lipemia</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>Tourniquet</td>
<td>Mastectomy Edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posture</td>
<td>Timing</td>
<td></td>
<td></td>
<td>Timing</td>
</tr>
</tbody>
</table>
Anticoagulants

- A substance that prevents blood from clotting. Yields a whole blood specimen or when spun a plasma specimen

**Types:**
- EDTA ethylenediaminetetraacetic acid in a tripotassium or disodium base (LAV)
- Sodium Citrate (Lt Blue)
- Heparin (Green)
- Potassium oxalate
  - Additive Sodium Fluoride
Additives

- Clot Activator
- Gel Separator

- Plain

Types of Specimens

- Whole Blood
  - Cells + Plasma
  - Anticoagulant prevents clotting
  - Mix specimen well
Types of Specimens

- **Plasma**
  - Plasma contains fibrinogen
  - Centrifuge whole blood, separate plasma from cells

- **Serum w/o activator**
  - Allow blood to clot for 20 - 30 minutes
  - With Activator 15- 20 minutes
  - Centrifuge 10 – 15 minutes, separate cells from serum
  - Serum does not contain fibrinogen
Tube Volume

- **Partially or over filled tubes**: filling additive tubes until the vacuum is exhausted is important for proper ratio of anticoagulant to blood
  - Coagulation studies: under filled tubes are not acceptable
  - CO2-under filled: Dec HCO₃
  - Blood Culture over filled: False
  - Positive

Short Draw Cause

- Push Back
- Tube was not placed correctly on the inside needle
- Tube has to be place straight into the holder
- Inside needle should be in the center of tube top
Short Draw Cause

- Tube was removed to quickly before the vacuum was exhausted
- Label on top and had a poor visual of the tube filling

Short Draw Cause

- Needle not in the vein properly
- Bevel is above the vein
- Bevel is below the vein
- Tube engaged before needle is completely in the vein
- Redirecting the needle
Short Draw Cause

- Tiny veins
- Removing the tourniquet too soon
- Poor circulation
- Veins that are hard
- Damaged veins

Short Draw Cause

- When using a butterfly a “Blank” or “Discard” tube should be drawn for Coagulation
- There is dead space in the butterfly tubing
- The vacuum pulls air into the tube and the correct fill is not obtained
Inverting the Specimens

- Invert the specimens that contain additives
- Poor mixing produce specimens with clots
  - EDTA
  - Sodium Citrate
- Micro clots sometimes go undetected
- CLSI recommends 5 – 10 inversions
  - Clot Activator tubes need to be inverted at least 5 times

Correct Order of Draw

- **Blood Cultures**: sterile specimen
- **Light Blue**: sodium citrate for coagulation. Tube should be full and well mixed
- **Serum Tube**: (with or without clot activator or gel separator)
- **Green**: heparin or plasma chemistry (with or without gel separator)
- **Lavender**: EDTA for Hematology
- **Gray**: oxalate/fluoride for glucose testing
Additive Contamination

- **Tests affected by EDTA contamination**
  - Calcium
  - PT
  - Potassium
  - APTT
  - Sodium
  - Serum Iron

- **Tests affected by Heparin**
  - PT
  - aPTT
  - ACT

- **Tests affected by Potassium Oxalate**
  - Potassium
  - RBC Morphology

Vein Selection

- **Antecubital Region**
  - Median Cubital
  - Cephalic
  - Basilic
  - Volar Venous network
    - (hand)
      - Basilic is the last choice and should not be used because of possible nerve damage
Specimen Processing

- Transportation
  - Deliver in a timely manner
- Processing
  - Tubes should be in upright at room temperature
- Separation
  - Prolonged contact of cells causes changes
- Centrifugation
  - Specimens should be fully clotted

Specimen Clotting

- Serum Specimens in Glass Tubes
  - 20 – 30 minutes
- Plastic Tubes
  - 30 – 45 minutes
- Clot Activators
  - 15 – 20 minutes
- Tubes should be upright at room temperature while clotting
Separation

- Plasma Specimens
  - One hour after collection
- Serum Specimens
  - Two hours after collection
- Prolonged contact with cells
  - Increase: CK, Lactate, LD, Ammonia
  - Decrease in glucose, Bicarbonate, Acid phosphatase

Plasma/Serum

- Heparinized plasma is preferred over serum for potassium tests
- When blood clots potassium is released from the cells into the serum
- Can falsely elevate the potassium results
Centrifugation

- Specimens should be fully clotted
- Always keep tops on tubes during centrifugation
- Balance correctly
- Centrifuge only once
  - Repeated centrifugation can cause hemolysis

Troubleshooting Hemolysis

Specimen Collection

<table>
<thead>
<tr>
<th>Error</th>
<th>Effect</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended tourniquet application</td>
<td>Over 1 minute causes hemoconcentration and potential cell rupture</td>
<td>Tourniquet should be on for one minute. Reapply after 2 minutes</td>
</tr>
<tr>
<td>Traumatic draw</td>
<td>Vein collapsed, probing causes cells to rupture</td>
<td>Avoid probing. If vein can not be located move needle slightly. Remove needle and begin again</td>
</tr>
<tr>
<td>Vigorous mixing of the tube</td>
<td>Mechanical trauma to the RBCs which caused then to rupture</td>
<td>Use appropriate number of inversions and gently invert the tube</td>
</tr>
</tbody>
</table>
### Specimen Collection

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<tr>
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<tbody>
<tr>
<td>Tube Fill Draw</td>
<td>Inappropriate ratio of blood to additive can cause cells to rupture</td>
<td>Fill tubes to the fill mark. Wait for the blood flow to cease prior to tube removal</td>
</tr>
<tr>
<td>Alcohol not dry</td>
<td>Residual alcohol can cause the RBCs to rupture</td>
<td>Allow the alcohol to dry for 15-30 seconds</td>
</tr>
<tr>
<td>Inappropriate tube vacuum</td>
<td>To much vacuum applied to a small or fragile vein</td>
<td>Select smaller tubes or the minimum amount needed</td>
</tr>
<tr>
<td>Needle Gauge</td>
<td>Too small a gauge cause the RBCs to be under a great force that will share them</td>
<td>Select a needle gauge for the vein size, location and condition</td>
</tr>
<tr>
<td></td>
<td>Too large gauge the blood enters to quickly</td>
<td></td>
</tr>
</tbody>
</table>

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<thead>
<tr>
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<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle Readjustment</td>
<td>Vein trauma may result when the needle placement is not accurate</td>
<td>The needle should be parallel to the vein. Avoid probing</td>
</tr>
<tr>
<td>Needle occlusion</td>
<td>May cause the blood to flow slowly and initiate RBC shearing</td>
<td>Needle bevel not in the correct position. Avoid rotating or changing needle position</td>
</tr>
<tr>
<td>Hematoma</td>
<td>Specimens collected by drawing blood through a hematoma may cause erroneous results</td>
<td>Select another site or collect distally to the hematoma</td>
</tr>
</tbody>
</table>
## Specimen Collection

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<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Syringe Collection</td>
<td>Too much force applied to the plunger. Pulling back too rapidly on the plunger creates excess force. Forceful transfer.</td>
<td>Move the plunger within the barrel for easy movement. Do not use a syringe size much larger than blood needed. Pull slowly and steadily. Use a transfer device.</td>
</tr>
<tr>
<td>Frothing of Blood</td>
<td>Extra force on the RBC membrane.</td>
<td>Use appropriate size needle and tube.</td>
</tr>
<tr>
<td>Cather Collection</td>
<td><em>Blood travels through several different diameters</em></td>
<td>Ensure all connections fit securely, collect discard tube, use small draw tubes, use transfer device when using a syringe.</td>
</tr>
</tbody>
</table>

## Specimen Transport

<table>
<thead>
<tr>
<th>Error</th>
<th>Effect</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive Temperature Fluctuation</td>
<td>Extreme temperature changes can cause cells to rupture.</td>
<td>Blood samples should be maintained at the appropriate temperature. Do not freeze RBCs.</td>
</tr>
<tr>
<td>Pneumatic Tube System</td>
<td>Hemolysis may be due to acceleration and or deceleration speed, length, angles and tube cushioning.</td>
<td>Systems should be validated. Ensure proper cushioning of tubes.</td>
</tr>
<tr>
<td>Time Delay</td>
<td>The longer plasma/serum is in contact with the cells, the greater the chance of intracellular changes.</td>
<td>Samples should be centrifuged within two hours of collection.</td>
</tr>
</tbody>
</table>
## Specimen Processing

<table>
<thead>
<tr>
<th>Error</th>
<th>Effects</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation</td>
<td>Excessive centrifugation or too much centrifugal force will cause cells to rupture</td>
<td>Samples should be centrifuged according to manufacturer’s recommendations</td>
</tr>
<tr>
<td>Rimming the Tube</td>
<td>Mechanical disruption of cell membranes with wooden applicator sticks to remove micro clots</td>
<td>Allow serum tubes to fully clot. Rimming should be avoided</td>
</tr>
<tr>
<td>Time Delay</td>
<td>Significant delays in processing can cause alteration in analyte levels</td>
<td>Processing of samples should be carried out in a timely fashion</td>
</tr>
</tbody>
</table>

## Specimen Storage

<table>
<thead>
<tr>
<th>Error</th>
<th>Effect</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma/Serum Contact</td>
<td>Red cells rupture as they age</td>
<td>Length of storage should not exceed manufacturer’s recommendations</td>
</tr>
<tr>
<td>Freezing</td>
<td>RBC’s should never be frozen. Formation of ice crystals cause them to rupture</td>
<td>Make sure serum /plasma is free of RBCs before freezing</td>
</tr>
<tr>
<td>Cooled specimens</td>
<td>Elevated electrolytes</td>
<td>Transport at room temperature</td>
</tr>
</tbody>
</table>
## Patient Factors

<table>
<thead>
<tr>
<th>Factors</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic disorders</td>
<td>May cause red cell lysis</td>
</tr>
<tr>
<td>Liver Disease</td>
<td></td>
</tr>
<tr>
<td>Sickle Cell Anemia</td>
<td></td>
</tr>
<tr>
<td>Autoimmune Hemolytic Anemia</td>
<td></td>
</tr>
<tr>
<td>Chemical agents</td>
<td>Depending on dosage, may cause red cell lysis</td>
</tr>
<tr>
<td>Lead</td>
<td></td>
</tr>
<tr>
<td>Antimalarial drugs</td>
<td></td>
</tr>
<tr>
<td>Sulfonamides</td>
<td></td>
</tr>
<tr>
<td>Physical Agents</td>
<td></td>
</tr>
<tr>
<td>Mechanical Heart Valve</td>
<td></td>
</tr>
<tr>
<td>Burns</td>
<td></td>
</tr>
<tr>
<td>Infectious Agents</td>
<td>Direct damage to RBCs</td>
</tr>
<tr>
<td>Parasites</td>
<td>Intravascular hemolysis</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
</tbody>
</table>

## Questions???

Thank You