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

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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

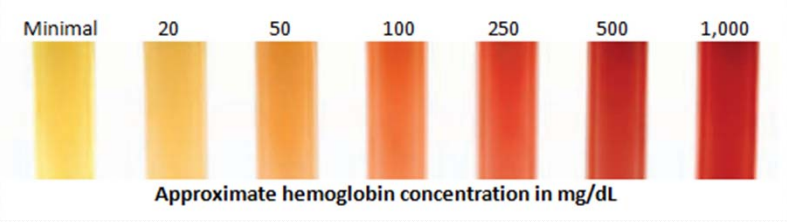
What is Hemolysis?

- 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
  - LD
  - Ammonia
  - Iron
  - Cholesterol
  - Sodium
  - Haptoglobin
  - Bilirubin
  - AST
  - Folate
  - Total Protein
  - Triglycerides
  - 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

Seriously Affected	Noticeably Affected	Slightly Affected
Potassium (K)	Iron	Phosphorus
Lactic Dehydrogenase (LD)	Alanine Aminotransferase (ALT)	Alkaline Phosphatase (ALP)
CBC	Thyroxine (T_4)	Total Protein (TP)
	PT	Albumin
	APTT	Magnesium (Mg)
	C-peptide	Calcium
		Rapid Plasma Reagin (RPR)

Hemoconcentration

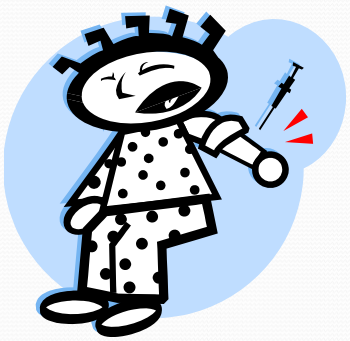
- Intravascular pressure build up, allows analytes to escape through the capillary walls and into the tissues
- This results with an concentration of analytes in the circulatory system
- Biochemical changes take place in the trapped blood

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

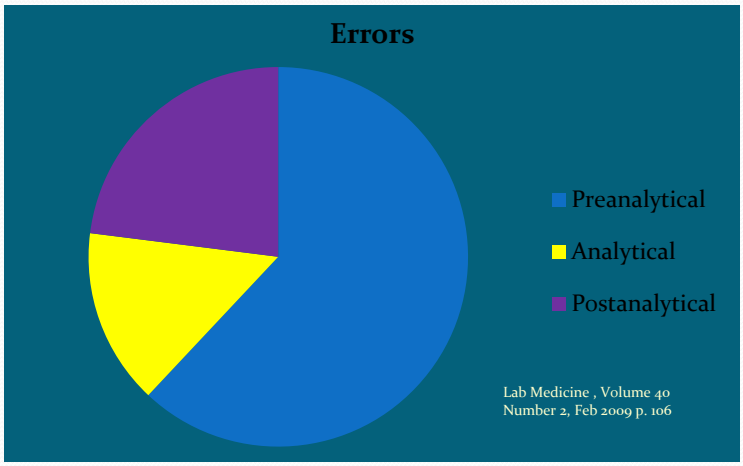
Ways to Prevent Hemoconcentration

- 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

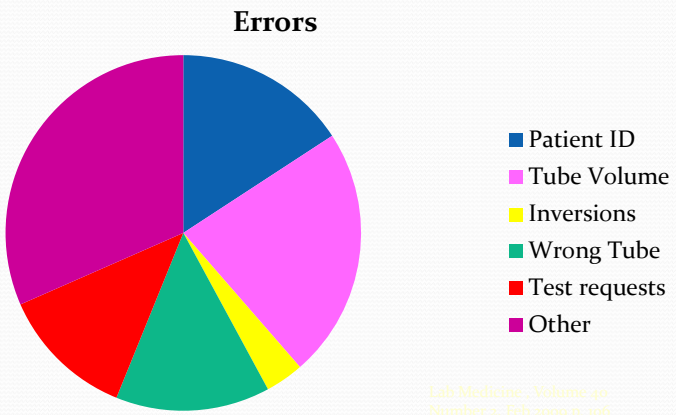
Rejected Specimens

- Preanalytical phase highly susceptible to error
- Hemolyzed specimens account for 60% of rejected specimens

Testing Errors



Pre Analytical Variables



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

Ordering	Collection	Collection	Processing	Transporting
Patient Identification Labeling Diet Exercise Posture	Additives Vein Selection Order of Draw Cleansing Tourniquet Timing	Specimen Volume Short Draw Inverting IV Lines and Line Draws Mastectomy Edema	Separation Centrifugation Hemolysis Lipemia	Light Evaporation Temperature Timing

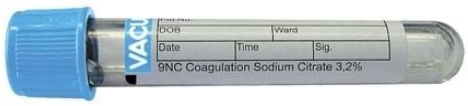
Anticoagulants

- 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

Anti - Coagulants

- EDTA

- Sodium Citrate

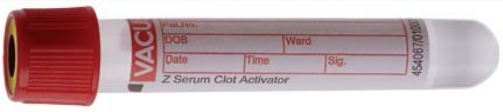


- Heparin

- Potassium Oxalate
  - Additive Sodium Fluoride

Additives

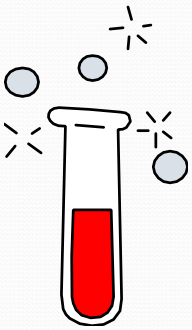
- Clot Activator
- Gel Separator



- Plain

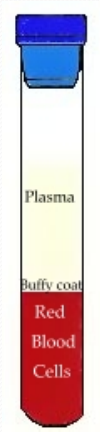
Types of Specimens

- **Whole Blood**
  - Cells + Plasma
  - Anticoagulant prevents clotting
  - Mix specimen well



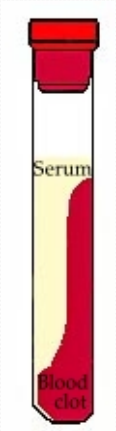
Types of Specimens

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



Types of Specimens

- **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

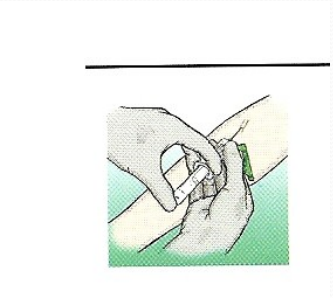
- **Partially or over filled tubes**: filling additive tubes until the vacuum is exhausted is important for proper ratio of anticoagulant to blood
  - Coagulation studies: under filled tubes are not acceptable
  - CO_2-under filled: Dec HCO_3
  - Blood Culture over filled: False

    Positive

Short Draw Cause

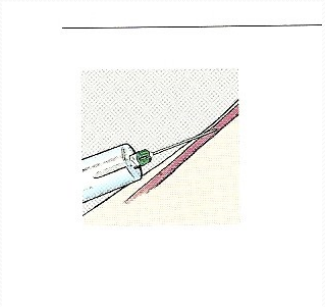
- Push Back
- Tube was not placed correctly on the inside needle
- Tube has to be place straight into the holder
- Inside needle should be in the center of tube top

Short Draw Cause



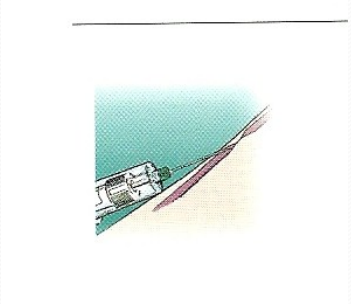
- Tube was removed to quickly before the vacuum was exhausted
- Label on top and had a poor visual of the tube filling

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 to 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

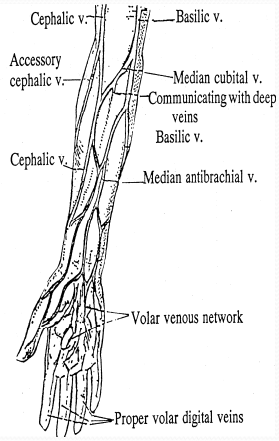
- **Blood Cultures**: sterile specimen
- **Light Blue**: sodium citrate for coagulation. Tube should be full and well mixed
- **Serum Tube:** (with or without clot activator or gel separator)
- **Green**: heparin or plasma chemistry (with or without gel separator)
- **Lavender**: EDTA for Hematology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

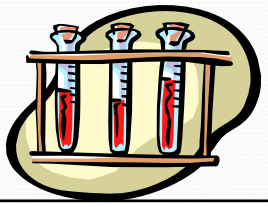
Vein Selection

- **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 temperat le 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

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

Specimen Collection

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

Specimen Collection

Error	Effect	Prevention
Needle Readjustment	Vein trauma may result when the needle placement is not accurate	The needle should be parallel to the vein. Avoid probing
Needle occlusion	May cause the blood to flow slowly and initiate RBC shearing	Needle bevel not in the correct position. Avoid rotating or changing needle position
Hematoma	Specimens collected by drawing blood through a hematoma may cause erroneous results	Select another site or collect distally to the hematoma

Specimen Collection

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

Specimen Transport

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

Specimen Processing

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

Specimen Storage

Error	Effect	Prevention
Plasma/Serum Contact	Red cells rupture as they age	Length of storage should not exceed manufacturer's recommendations
Freezing	RBC's should never be frozen, Formation of ice crystals cause them to rupture	Make sure serum /plasma is free of RBCs before freezing
Cooled specimens	Elevated electrolytes	Transport at room temperature

Patient Factors

Factors	Effects
Metabolic disorders Liver Disease Sickle Cell Anemia Autoimmune Hemolytic Anemia	May cause red cell lysis
Chemical agents Lead Antimalarial drugs Sulfonamides	Depending on dosage, may cause red cell lysis
Physical Agents Mechanical Heart Valve Burns	Direct damage to RBCs Intravascular hemolysis
Infectious Agents Parasites Bacteria	Fragility of RBCs increase

Questions???

Thank You