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TITLE: Utility of HIL in Clinical Chemistry

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Slide 1:

Hello, my name is **Yachana Kataria**. I am a clinical chemistry fellow at Boston Children's Hospital. Welcome to this Pearl of Laboratory Medicine on "**Utility of HIL in Clinical Chemistry**"

Slide 2:

- Hemolysis, icterus, & lipemia also commonly known as HIL are the most common specimen integrity issues that can interfere with laboratory tests and may lead to erroneous results and interpretations and ultimately to inappropriate medical decisions.
- HIL indices are an objective way to detect interferences compared to the traditional practice of visual inspection.
- Visual inspection is subjective, unreliable, and time consuming.
- Whereas, measurement of serum indices are available on most modern day chemistry analyzers and provide a standardized and reproducible tool to estimate interferences. This subsequently improves quality, efficiency and uniformity of the laboratory testing process.

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- While there are definitive strengths of HIL serum indices, it is important to recognize the limitations of HIL detection on automated analyzers as well.
- On most chemistry auto-analyzers, hemolysis and icterus is detected by spectrophotometry.
- Hemoglobin absorbs light at wavelengths between the ranges of 340 to 440 nm and between 540 to 580 nm.

- Bilirubin absorbs light at wavelengths between 400 and 500 nm.
- Lipemia causes light scattering that subsequently effects the measurements of assays utilizing nephelometric and turbidimetric methods.
- There is an overlap in the spectra of hemoglobin, bilirubin and lipemia. While there is no currently defined best method for measuring serum indices, manufactures' product inserts may provide useful information on how the interferences were assessed.
- More than one HIL interferents maybe simultaneously present in a patient sample. The presence of one HIL interferent may adversely affect the measurement of another HIL interferent.
- Non-HIL interferents may also be present. Examples of such interferents include methemalbumin, beta-carotenes, dyes and contrast media present in the blood.
- Lastly, HIL indices do not replace standard assays of hemoglobin, bilirubin, or triglycerides since it's a non-specific semi-quantitative method.

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- Analyzer manufacturers perform interference testing to assess potential HIL interferences on analytes.
- Various degrees of hemolysis, icterus and lipemia are tested to determine if the result of an assay is significantly altered.
- Based on acceptability criteria of interferences, the manufacturer defines a cut off value.
- In the example shown here, response of the ABC analyte is not affected by increasing bilirubin concentrations.
- However, the same does not hold true for analyte DEF. The DEF response changes with increasing bilirubin concentrations.

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- Hemolysis index (H) is assessed by the amount of red pigmentation associated with free hemoglobin.
- Upon damage to the cell membrane, hemoglobin and other intracellular components from erythrocytes are released into the extracellular space of blood.

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- Here is a list of analytes that are commonly affected by hemolysis in the lab. This is by no means a comprehensive list and you should always refer to the product manual utilized in your lab.
- Release of analytes found in high concentrations in red blood cells will be falsely elevated. Examples of such analytes include, potassium, magnesium, phosphate, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST).
- Hemoglobin binds to haptoglobin, therefore haptoglobin exhibits a negative interference on the measured haptoglobin concentration.
- Presence of hemolysis also causes a negative or positive interference on the Troponin T and I assays. The degree and direction of the interference is method dependent. For example, hemolysis falsely decreases concentrations of cTnT assays on the Roche instruments, where as cTnI measured assay on the Vitros 5600 is falsely increases in hemolyzed samples. Refer to your manufacturer guidelines to determine how your laboratory troponin assay is affected by hemolysis.
- The actual mechanism of hemolysis interference in troponin assays is not well understood. Experimental evidence suggests that release of proteases from red blood cells degrade the antigenic region of the cTnT assays thus preventing them to be detected.
- Additionally, release of erythrocytes' intracellular fluid may also causes dilution of analytes found at low concentrations in the erythrocyte.

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- The way to correct hemolysis largely depends on the analyte and the instruments in your laboratory.
- One way to deal with a hemolyzed specimen is to first determine if the H index is above the hemolysis cut-off limit for the analyte of interest.
- If the H-index is below the cut-off, you may proceed with testing. However, if the H-index is above the cut-off limit, determine whether you are able to dilute the sample by referring to the manufacturer guidelines.
- If dilution is acceptable you may proceed to testing after diluting the specimen.
- If dilution is not acceptable, determine whether it is in-vitro or in-vivo hemolysis. This can be accomplished by measuring haptoglobin. Decreased concentrations of haptoglobin

are a pronounced and is a specific effect in in-vivo hemolysis. Whereas, haptoglobin levels remain unchanged in the in-vitro hemolysis.

- In instances of in-vitro hemolysis, the specimen should be rejected and the clinical staff ought to be notified to recollect the sample properly.
- In instances of in-vivo hemolysis, the clinical staff ought to be notified and the underlying pathology should be addressed. As the lab professional, you can also recommend alternative testing. For example, you can recommend measurement of ALT as oppose to AST to help evaluate liver function.

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- Icteric (I) index is a measure of the yellow pigmentation of the sample due to increased bilirubin concentration.
- Bilirubin exists in unconjugated and conjugated forms. They are both thought to equally contribute to the interference.
- Elevated bilirubin concentrations could be the result of hepatic diseases, hemolytic disorders, and other obstructive biliary disorders.

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- Bilirubin has been shown to produce a negative bias on assays that use sequential oxidase and peroxidase enzymatic reactions. For example, bilirubin produces a negative bias on common assays for cholesterol, glucose and triglycerides.
- Bilirubin also produces a negative bias for the Jaffe creatinine method. The Jaffe method involves the reaction of creatinine and picric acid in alkaline conditions. The absorbance of the formed complex is subsequently measured at 520 nm.
- Bilirubin inhibits the reaction between creatinine and alkaline picrate. Bilirubin under alkaline conditions is oxidized to biliverdin thereby causing a decrease in absorbance.

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- One way to deal with an icteric specimen is to first determine if the I-index is above the icterus cut-off limit for the analyte of interest.

- If the I-index isn't, you may proceed with testing. However, if the I-index is above the cut-off limit, determine whether you are able to dilute the sample by referring to the manufacturer guidelines.
- If dilution is acceptable, you may proceed to testing after diluting the specimen.
- If dilution is not acceptable, you will have to explore alternative methods.
- An example is to utilize an enzymatic method rather than the Jaffe method for creatinine measurement.

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- Lipemic index (L) is assessed by turbidity due to elevated lipoproteins.
- Lipoproteins that are the primary cause of turbidity are triglyceride-rich lipoproteins such as VLDL and chylomicrons.
- Turbidity can be also caused by erythrocyte debris, platelets, leukocytes, fibrin clots or contaminating particulate matter.
- The degree of scattering depends on the number, size, and refractive index of the suspended particles.
- Patient samples contain a mixture of various particle sizes. The sample appears lipemic because light is scattered at all angles.

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- Lipemia can also interfere by causing water displacement in plasma.
- Normal human plasma consists of approximately 93% of water and 7% of proteins and lipids.
- Most laboratories utilize indirect ISE to quantitate electrolytes. In this method, the plasma is first diluted with an aqueous diluent before the electrode measurement is taken. This method makes the assumption that the sample is composed of 93% water.
- In a lipemic sample, as the lipid percentage increases the proportion of water in the sample decreases. Dilution of the sample in the aqueous diluent before indirect ISE measurement results in an over-dilution effect because of less water. Ultimately concentration of electrolytes will be falsely decreased.
- This phenomenon is commonly known as the electrolyte exclusion effect.

- The example on the slide is for a hypothetical lipemic specimen where the sodium concentration would be falsely reported as 126 mmol per liter from an indirect ISE method.

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- There are multiple ways to handle lipid interference. One approach is to first determine the cause of lipemia.
- If the cause is due to endogenous reasons such as hypercholesterolemia. There are multiple ways to remove the lipids. You can either dilute the sample if acceptable by the manufacture guidelines.
- Alternatively, you can ultracentrifuge the specimen and remove the top lipid layer and subsequently measure the analyte of interest in the infranatant.
- Steroids and some drugs such as valproic acid are found in the lipid layer. In such cases, removing the lipid fraction is unacceptable. These samples should be diluted to attenuate the interference while still measuring the analytes in the analytical linear range of the method.
- Lipid clearing agents can also be added and subsequently centrifuged. Upon centrifugation, these particles precipitate to the bottom of the tube and the analyte is measured in the supernatant.
- As mentioned earlier, for analytes measured by indirect ISEs, you can utilize direct ISEs.
- If the cause of lipemia is due to exogenous reasons, such as TPN administration. It is recommended to ask the clinical staff to redraw the specimen following appropriate guidelines to reduce contamination.

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- In conclusion, automated assessment of hemolysis, icterus, & lipemia (HIL) provides the laboratory a standardized, reproducible and efficient tool to detect possible interference related to sample integrity.

Slide 15: References

1. Clinical and Laboratory Standards Institute (CLSI) ” Hemolysis, icterus, and lipemia/turbidity indices as indicators of interference in clinical laboratory analysis;

Approved guideline." *CLSI document C56-A, Wayne, Clinical and Laboratory Standards Institute* (2012).

2. Dimeski, Goce. "Interference testing." *The Clinical Biochemist Reviews* 29.Suppl 1 (2008): S43.
3. Nikolac, Nora. "Lipemia: causes, interference mechanisms, detection and management." *Biochemia medica* 24.1 (2014): 57-67.
4. Bowen, Raffick AR, et al. "Impact of blood collection devices on clinical chemistry assays." *Clinical biochemistry* 43.1 (2010): 4-25.

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org

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