

INTERFERENCE TESTING

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Interference Testing

- Interference occurs when an endogenous or exogenous substance alters an assay result.
- Manufacturers are required to perform interference testing during assay development.
- Laboratories may choose to verify these claims as part of Good Laboratory Practice or while investigating questionable results.

Common Interferents

- Lipemia (triglycerides, turbidity)
- Hemolysis (hemoglobin and RBC contents)
- Proteins (γ -globulins, monoclonal gammopathies)
- Icterus (bilirubin, conjugated and unconjugated)

Lipemia

- Turbidity of a sample caused by elevated triglycerides (mostly chylomicrons +/- VLDL) which is visible to the naked eye.
- Caused by recent dietary fat intake, infusion of lipids (parenteral or enteral nutrition), or abnormal lipid metabolism.
- Fasting is recommended to decrease the chance of lipemia associated with recent food intake.
- Other causes of turbidity: cold agglutinins, monoclonal gammopathies, heparin therapy.

Lipemia

- Turbidity is typically visible when TRIG > 300 mg/dL.
- The extent of turbidity can be monitored by absorbance at wavelengths > 600 nm.
- Lipemia interferes with nearly all photometric measurements by absorbance and light scattering.
- Sample blanking and bichromatic readings help decrease effect but do not eliminate.

Lipemia: Volume Depletion (Matrix) Effect

- Apparent concentration of analyte is decreased because lipoproteins decrease the available water (volume taken up by lipoproteins is included in the analyte concentration calculation).
- Seen with flame photometry and indirect ISE but not with direct potentiometry.
- Analyte may not be accessible to reagent, e.g. antigen may not interact with antibody.

Lipemia

To prevent lipemia:

- 12 hour fast/ Stop parenteral feeding for 8 hours.
- Remove lipemia/turbidity (after measurement of triglycerides, cholesterol, and apolipoprotein B, etc.):
 - Centrifugation
 - Extraction of lipids with organic solvents
 - Precipitation of lipoproteins with polyanions, cyclodextrins, or polyethylene glycol
 - Delipidation with detergents, lipase, deoxycholate, etc.
 - Report method used for removal of turbidity and effect on specific analytes. Document that process does not interfere with measurements.

Lipemia

- For interference testing, lipemia is often simulated using a 10% to 20% fat emulsion (Intralipid).
- However, there are significant differences in the analytical response to these synthetic emulsions and “physiological” lipemia.
- Best to use intact human lipoproteins, both chylomicrons and VLDL.

Hemolysis

- Hemolysis can occur in the patient (*in vivo*) or outside the patient (*in vitro*) preanalytically.
- Interference resulting from hemolysis can be caused directly by hemoglobin absorbance or by leakage of RBC constituents, such as K, AST, LDH, etc.
- Hemoglobin >30 mg/dL is visually detectable.
- CLSI recommends testing up to 500 mg/dL hemoglobin by spiking hemolysate into serum pool.
- Testing does not differentiate between *in vivo* and *in vitro* hemolysis.

In Vivo vs. *In Vitro* Hemolysis

- Free Hb *in vivo* rapidly binds to haptoglobin and the complex is catabolized.
- A decrease in haptoglobin is a characteristic of *in vivo* hemolysis.
- Increases in bilirubin and reticulocyte counts are also characteristic of *in vivo* hemolysis.
- During *in vitro* hemolysis, all RBC constituents with ~10-fold higher intracellular concentration are increased, while haptoglobin concentration is normal.
- About 3% of hemolysis is *in vivo*.

In Vivo vs. *In Vitro* Hemolysis

<i>In-vivo</i> Hemolysis	<i>In-vitro</i> Hemolysis
↓ Haptoglobin	– Haptoglobin
↑ Unconjugated Bilirubin	↑ Hemoglobin
↑ Reticulocytes (response to anemia)	↑ AST
↑ LDH	↑ LDH
	↑ K

Given the clinical significance of hemolysis, each hemolyzed sample should be documented and the cause investigated. Procedures for handling and reporting hemolyzed samples should be available, including rejection criteria.

When all samples from a patient are hemolyzed, *in-vivo* hemolysis should be suspected, and reported to the physician.

Hemolysis

Blood cell constituents can directly and indirectly interfere with certain assays:

- Hb “pseudo”-peroxidase activity may interfere with bilirubin measurement by inhibiting diazonium color formation and may inhibit other reactions: ALP, Fe, Lipase, GGT, Na, Cl.
- Hb can increase absorbance value (method and analyte-dependent).
- Artifactual release of intracellular contents may affect many test results: K, LDH, Mg, P, AST, ALT.
- Proteases can decrease activity of coagulation factors.

Causes of Hemolysis

In Vivo

- Extravascular:
 - Enzyme deficiencies (e.g. glucose-6-phosphate dehydrogenase)
 - Hemoglobinopathies (e.g. sickle cell, thalassemia)
 - Infection
 - Autoimmune hemolytic anemia
 - Hypersplenism
 - Liver disease
- Intravascular:
 - Microangiopathy
 - Transfusion reaction
 - Infection
 - Paroxysmal cold hemoglobinuria
 - Paroxysmal nocturnal hemoglobinuria

In Vitro

- Excessive aspiration force during phlebotomy
- Catheter partially obstructed
- Blood forced into tube through syringe
- Specimen frozen
- Mechanical damage (e.g. shaking, pneumatic tube transport)
- Delay in analysis

From: Cook SL, Bruns DE.
Clin Chem 2012; 58:974.

Hemolysis

- Interference is method specific.
- In some cases interferent can be reduced or eliminated by pretreatment:
 - Use alternate assay that is not sensitive to interferent
 - Deproteinization or molecular sieving may help
- Modern approach
 - Sample blanking and bichromatic monitoring decrease the absorbance effect- but not the effect of RBC contents
 - Ultrafiltration with multilayer film technology reduces interference.

Icterus

- Bilirubin occurs in serum as relatively insoluble free molecule, as water soluble conjugate (mono- and di-glucuronides), and covalently bound to albumin.
- Ditaurobilirubin is often used as a surrogate for conjugated bilirubin in manufacture of calibrators and controls and for interference testing.
- Conjugated bilirubin is often seen in urine when present in high concentration in serum.
- Bilirubin causes photometric interference because of its strong absorbance between 340 and 500 nm.

Icterus

- The linear range of assay can be reduced due to high background absorbance.
- Common assays with icteric interference:
 - Jaffe
 - Phosphate (molybdate)
 - Oxidase/peroxidase reactions: glucose.
Cholesterol, triglycerides, uric acid, creatinine
 - Albumin dye- binding assays
- Visual inspection of samples is NOT sensitive.

Icterus

- UV monitoring at 450 – 575 nm is used to assess bilirubin, but carotenoids and other compounds may interfere.
- The high prevalence of hyperbilirubinemia in hospital patients can be challenging: (ICU, gastroenterology, surgical, pediatrics), and make assessment of bilirubin interference and choice of assays especially important.
- Blanking procedures are useful; kinetic reactions better than endpoint.

Icterus

- Additives may reduce or eliminate bilirubin interference, e.g. bilirubin oxidase improves creatinine assay.
- Ultrafiltration helps reduce interference because bilirubin binds to protein. A 20 kDa cutoff provides a protein-free filtrate.
- Consider alternate methods, such as immunoassay for albumin.

Interference Summary

- Blanking will not completely eliminate chemical interference.
- It is never appropriate to “mathematically correct” for interference.
- Lab must understand assay limitations and interference thresholds so that inaccurate results are not reported.
- Good Laboratory Practice is not to simply rely on the manufacturer. What is the performance of your assay system in your laboratory?

Interference FAQs

CAP AGC 24130. “Are common interferences evaluated for all analytes measured with each reagent system, or is credible information available?”

CAP Answer:

“We do not require each laboratory to conduct an exhaustive study for interferences. If there is manufacturer-supplied information, you must carefully review that material and judge its relevance/acceptability for your setting...”

www.cap.org



Interference FAQs

James Westgard, Method validation: frequently asked questions

“Why is it necessary to validate method performance when the manufacturer has already performed extensive studies?”

Answer:

“It is important to demonstrate that the method performs well under the operating conditions of your laboratory, and that it provides reliable test results for your patients. There are many factors that can effect method performance such as different lots of calibrators and reagents, changes in supplies and suppliers of instrument components, changes in manufacturing from the production of prototypes to final field instruments, effects of shipment and storage, as well as local climate control conditions, quality of water, stability of electrical power, and of course, the skills of the analysts...”

www.westgard.com/basic-method-validation-3rd-edition-faqs.htm



Key References

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