Academic Pathology: Volume 6
DOI: 10.1177/2374289519888754
journals.sagepub.com/home/apc

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Educational Case: Hemolysis and Lipemia Interference With Laboratory Testing

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.[1]

Keywords
pathology competencies, diagnostic medicine, assay interference, preanalytical error, hemolysis, hypertriglyceridemia, lipemia, clinical chemistry tests

Received May 31, 2019. Received revised August 26, 2019. Accepted for publication October 12, 2019.

Primary Objective

Objective GP1.5: Test Variability. Explain the difference between technical variability and biologic variability including how physical and chemical parameters, such as sample size, hemolysis, and lipemia, can affect test results. Define analytical uncertainty, precision, accuracy, and coefficient of variation, and describe factors that contribute to each.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic GP: General Principles; Learning Goal 1: Laboratory Tests.

Patient Presentation

A nurse in the emergency department (ED) of a large hospital contacts the clinical laboratory due to tests canceled due to hemolysis or lipemia in 2 unrelated patients. The first patient was a 58-year-old man with chest pain who had tests for potassium and troponin T canceled due to hemolysis (Table 1). The second patient was a 35-year-old female with history of hypertriglyceridemia and multiple prior admissions for acute pancreatitis. This patient also had potassium and troponin T canceled due to hemolysis; in addition, testing for alanine aminotransferase and aspartate aminotransferase were canceled due to lipemia (Table 1). Per institutional policy, the clinical laboratory called the ED regarding the test cancellations. The electronic medical record (EMR) also displayed "Hemolyzed" or "Lipemic" as the reason for cancellation. The ED nurse wants to know if there is anything they can do to prevent further cancellations of tests.

Diagnostic Findings

Selected laboratory studies from the ED for the 2 patients are shown in Table 1. The test results given in Table 1 reflect multiple tests which had been canceled due to either hemolysis or lipemia. The laboratory tests for patient 2 support the diagnosis of hypertriglyceridemia and acute pancreatitis, with markedly elevated amylase and lipase. Figure 1 shows plasma for the 2 patients (patient 1 on left, patient 2 on right).

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Table 1. Laboratory Test Results.

Lab Tests Performed on Plasma	Patient 1	Patient 2	Normal Range
Sodium	139	142	135-145 mEq/L
Potassium	Hemolyzed	Hemolyzed	3.5-5.0 mEq/L
Chloride	99	101	95-107 mEq/L
CO_2	25	22	22-29 mEq/L
Blood urea nitrogen	17	9	10-20 mg/dL
Creatinine	0.8	0.5	0.6-1.2 mg/dL
Glucose	96	171	65-139 mg/dL
Calcium	10.2	9.1	8.5-10.5 mg/dL
Troponin T	Hemolyzed	Hemolyzed	<0.03 ng/mL
Amylase	Not performed	1320	30-110 U/L
Lipase	Not performed	2255	5-208 U/L
Alanine aminotransferase	Not performed	Lipemic	<34 U/L
Aspartate aminotransferase	Not performed	Lipemic	<32 U/L
Triglycerides	Not performed	1852	<200 mg/dL

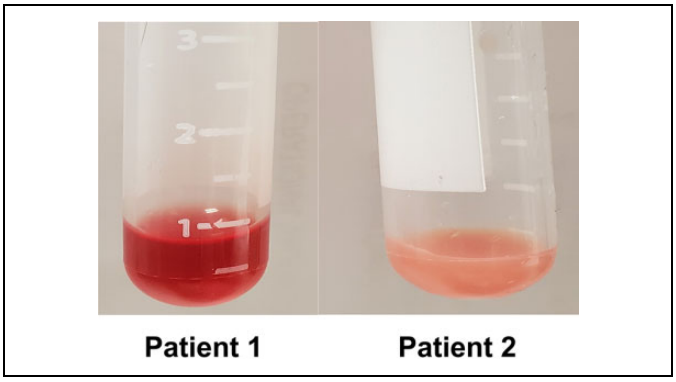

Figure 1. Plasma from patient 1 (left) shows severe hemolysis. Plasma from patient 2 (right) is lipemic (turbid and opaque) and also slightly hemolyzed.

Questions/Discussion Points

What Are the Most Likely Explanations of the Hemolysis and Lipemia?

The plasma for patient 1 visually shows the presence of hemolysis (Figure 1, left). As will be discussed below, the most likely explanations involve phlebotomy technique, although there are many other possibilities. The plasma for patient 2 shows a mixture of hemolysis and lipemia (Figure 1, right). The specimen is turbid from lipemia and also has a pink tinge from hemolysis. The most likely explanation for lipemia in this patient is a medical condition causing severe hypertriglyceridemia, as has been previously documented for this patient. The lipemia could be worsened by recent ingestion of food with high lipid content.

What Are the Main Causes of Hemolysis in Blood Tubes?

Hemolysis refers to lysis of red blood cells (erythrocytes).[2,3] Plasma or serum is normally clear (ie, not turbid or cloudy) and straw colored. In specimens obtained by venipuncture, hemolysis is visually evident by pinkish or reddish color of plasma or serum that has been separated from erythrocytes. Many variables impact hemolysis of a blood specimen.[4] In terms of frequency, the most common factors are associated with phlebotomy technique and sample processing/transport (in vitro factors). These include use of narrow gauge needles, forceful use of syringe, overly vigorous shaking of blood tube, improper specimen transport temperature, delay in separating serum/plasma from cells, and suboptimal centrifugation force and/or time. A variety of disease processes can result in hemolysis such as hemolytic anemias, hemoglobinopathies, sepsis, and parasite infections (in vivo factors). In vitro factors are influenced by experience and skill of the person doing phlebotomy and also by adherence to good practices with regard to specimen transport and processing.

What Are the Main Causes of Lipemia in Blood Tubes?

In contrast to hemolysis, lipemia occurs based on the lipid content (especially triglycerides) of the drawn blood and is not dependent on factors associated with the phlebotomist.[5] Lipemia makes plasma or serum turbid and opaque. In the absence of other color interferences, lipemic plasma/serum will appear milky white. The most common cause of lipemia is that the patient is not fasting and has eaten close in time to the blood draw.[4] This effect is most dramatic when the patient has consumed a meal with high fat content. However, nonfasting on its own usually does not result in enough lipemia to significantly impact laboratory tests. The more challenging cases occur when the lipemia is severe enough to interfere with laboratory testing. Based on retrospective studies, severe lipemia capable of causing significant interference with laboratory testing can occur either with disease processes associated with hypertriglyceridemia (eg, diabetes mellitus, genetic hyperlipidemias) or recent intravenous infusion with lipid emulsions as used for parenteral nutrition or some poorly water-soluble drugs (eg, the general anesthetic propofol).[6]

How Does Hemolysis Impact Laboratory Testing?

Hemolysis affects laboratory testing by 3 main mechanisms.[3,7] First, lysis of erythrocytes releases intracellular constituents such as aspartate aminotransferase, lactate dehydrogenase, and potassium, resulting in falsely elevated concentrations of these analytes. These tests are among the most sensitive to hemolysis and are also frequently ordered, making them the most common analytes to be canceled due to hemolysis. Second, hemolysis releases proteases from erythrocytes that can degrade proteins such as insulin and cardiac troponin, resulting in falsely lower concentrations. Third, the presence of excess hemoglobin and other constituents in the plasma/serum (noticeable by the color change) can interfere with spectrophotometric measurements. The degree of impact will vary depending on the exact analytical methodology and wavelength use for spectrophotometric determination for the assay.

How Does Lipemia Impact Laboratory Testing?

Lipemia results from sample turbidity from accumulation of lipoprotein particles and can interfere with laboratory analysis by several mechanisms.[5,8] First, lipemia can increase absorption of light and thereby decrease light transmittance used for spectrophotometric analysis. Second, lipemia can cause volume displacement, especially impacting analysis of electrolytes. Third, lipemic specimens can be nonhomogeneous, causing issues with how automated laboratory analyzers sense sample volume and pipette/aliquot specimens. These effects can result in analyzers erroneously determining that insufficient sample is present or in pipetting inaccurate volumes of specimen. Lastly, severely lipemic specimens are more prone to hemolysis.[6,9] The mechanism behind this effect is not completely understood, but one theory is that lipids act as detergents that disrupt erythrocyte membranes as the samples are processed and centrifuged. The end result of this phenomenon, however, can be a specimen that is massively hemolyzed and lipemic and practically unanalyzable for some laboratory tests.

How Are Hemolysis and Lipemia Detected and Quantified?

The simplest method for assessing hemolysis and lipemia is visual inspection, a practice still common in smaller clinical laboratories and/or those without instrumentation to detect these interferences.[3,8] The visual approach is limited by significant inaccuracy and interindividual variation in interpretation. For hemolysis, subtle color differences in specimens can be hard to distinguish, especially if other factors like lipemia are present. For lipemia, the degree of turbidity is visually difficult to resolve; beyond a certain point, the human eye does not readily perceive differences as the plasma/serum simply appears turbid and opaque. Measurement of triglycerides in plasma or serum can roughly approximate degree of lipemia, but the relationship between triglycerides and turbidity is complex and nonlinear.[5,8,9] In laboratories performing visual inspection of samples, hemolysis and lipemia are often graded on a simple scale such as absent, mild, moderate, and severe.

An alternative approach is done by many clinical chemistry analyzers that rapidly assess hemolysis and lipemia "indices" using spectrophotometric analysis.[5,8,10,11] Common wavelength ranges are approximately 570 to 600 nm for hemolysis and 660 to 700 nm for lipemia, with many instruments assessing 2 or more wavelengths in these ranges. These analyzers also commonly assess a third index for icterus caused by presence of bilirubin and similar constituents in plasma or serum.

There are advantages and disadvantages to automatic detection of hemolysis and lipemia indices.[8,10,11] These methods are rapid, reproducible, and provide a quantitative index. As an example, a hemolysis index of less than 30 may indicate minimal hemolysis, 100 may indicate mild hemolysis, and greater than 400 indicate severe hemolysis. These quantitative values can be used for computer rules that determine what happens to results from a specimen (eg, cancel, report with warnings). For instance, manufacturer data provided in the assay package insert for potassium may indicate that significant interference occurs above a specific hemolysis index. This threshold can be used for actions taken by the clinical laboratory.

How Should Results From Samples With Hemolysis or Lipemia be Reported?

A number of options exist for reporting of hemolyzed or lipemic specimens (summarized in Table 2). The first is simply to cancel the testing potentially impacted by interference, with documentation of the reason for cancellation. This option is influenced by EMR functionality with respect to displaying laboratory results and comments/warnings. Ideally, the cancellation reason should be easily evident and not just a generic

Table 2. Approaches to Reporting Testing in Specimens With Hemolysis or Lipemia.

Approach	Potential Advantages	Potential Drawbacks/Challenges
Cancel testing, suppress test result	• Prevent erroneous results • Facilitate collection of new specimen	• Risk of missing clinically significant abnormality (eg, hyperkalemia) • Some specimens may be irreplaceable • Patient may be unavailable for recollection (eg, outpatient who has already traveled back home)
Provide warning on interference but release test result	• May alert clinical team to clinically significant abnormality • Allows clinical team to make judgment on test result	• Warning on interference may not be understood or noticed • Can be difficult to predict magnitude of interference, with potential for erroneous interpretation
Release test results only within specified interference range	• Releases tests results with lower likelihood of significant interference impact • Can be customized for selected tests with higher clinical urgency (eg, potassium, troponin)	• Often requires more data on test interference than reported in package inserts • Relationship between level of hemolysis or lipemia and impact on tests results can be complicated and variable
Contact clinical service if interference present (may be combined with any approach above)	• Can allow for timely recollection, if indicated	• Time and effort involved in contacting clinical staff, who may be unreachable or for whom information is not helpful

designation of "cancelled" without clear explanation of the reason. The second option is to report the test results (eg, potassium of 4.6 mEq/L) with some warning or comment related to the presence of the potential interference. A more sophisticated third approach would be to report test results only within a specific range of interference (eg, a range of mild hemolysis) but cancel if the interference is more profound. The clinical laboratory also needs to have policies related to whether the clinical team is contacted (eg, by phone, pager, etc) when specimens are suboptimal. Communication with the clinical team can facilitate recollection of specimens, if indicated.

There are potential pitfalls with any of these approaches (Table 2), as can be illustrated using serum/plasma potassium. As noted above, potassium is sensitive to hemolysis, which can produce falsely elevated levels by the release of intracellular potassium from lysed erythrocytes. Canceling potassium testing due to hemolysis carries the risk of missing a serious abnormality in potassium. For example, a patient may actually have hyperkalemia that could be missed if hemolysis was also present and led to test cancellation. In this scenario, reporting the numeric potassium concentration and also indicating hemolysis in the specimen may allow the clinical team to recognize the potential for actual hyperkalemia.

However, reporting a result for a hemolyzed specimen, even with a disclaimer or warning, carries the risk that clinical actions may be taken based on an erroneous result. For instance, hemolysis may cause a patient with normal plasma potassium to have a specimen with potassium in the hyperkalemia range. Clinical actions to lower the potassium (eg, dialysis, medications) would be unnecessary and potentially dangerous as there is no abnormality. Hemolysis also mask hypokalemia by elevating potassium to within the reference range, with no abnormal result flags other than the hemolysis warning. Ideally, if clinical decisions are to be based on a laboratory value that might be significantly impacted by hemolysis, obtaining a new specimen is highly recommended. This can be especially important for assays such as cardiac troponin that drive time-sensitive clinical decisions. One additional consideration is that the magnitude and direction of interference is not always well defined. While some tests such as potassium show a consistent effect to increasing sample hemolysis, other tests may show a decreased response or even more complicated biphasic patterns. Table 2 summarizes potential advantages and pitfalls/drawbacks to various means of reporting specimen interference.

How Does Hemolysis and Lipemia Impact Testing Using Whole Blood?

It is also important to note that automated detection of hemolysis and lipemia is currently most robust for plasma and serum specimens. Detection of hemolysis and lipemia is technically more difficult for whole-blood samples (not centrifuged) that are used as the specimens for blood counts, blood gas analyzers, and some point of care and smaller chemistry analyzers.[12] In particular, the methods used for automated detection of hemolysis in plasma or serum will not work in whole-blood specimens which contain erythrocytes that heavily absorb in the wavelengths used to assess presence of hemolysis. This can be an issue in that these type of analyzers may be used for measurement of analytes such as potassium and aspartate aminotransferase that are significantly impacted by presence of hemolysis. Laboratory and point-of-care devices that analyze whole-blood specimens may have more crude detection for hemolysis or lipemia including semiquantitative interference indices or warning alarms for high absorbance or turbidity.

How Can Hemolysis Impact on Laboratory Testing be Avoided or Minimized?

One initial approach to figuring out cause of hemolysis is to see whether hemolysis is occurring with multiple staff doing the phlebotomy. Some institutions utilize the EMR and/or laboratory information system to document the person performing the phlebotomy and sometimes additionally the method of obtaining blood (eg, standard venipuncture, draw from intravenous line, etc). This type of electronic record facilitates investigations into hemolysis. At other institutions, information on phlebotomist may only be accessible on paperwork or initials on specimen containers.

Phlebotomy technique is the most common factor involved in hemolysis. Some studies have shown that phlebotomy technique accounts for 50% to 75% of explanations for sample hemolysis. If all specimens for patient have so far been obtained by a single staff member (eg, nurse or phlebotomist in the ED), then a logical next step is to have someone experienced with phlebotomy obtain a specimen to see if that resolves the problem. Proper specimen technique can be reviewed with the first staff member. Poor technique may be quickly evident when one person has multiple patients with hemolyzed specimens. If the personnel performing the phlebotomy is recorded in the EMR, tracking specimen hemolysis rates is a quality metric and a powerful tool to minimize sample hemolysis.

How Can Lipemia Impact on Laboratory Testing be Avoided or Minimized?

The clinical laboratory management of lipemic specimens is not as well standardized as for hemolyzed specimens. The simplest recommendation is to encourage fasting collection. If the lipemia is likely due to recent intravenous infusion of a lipid emulsion, then phlebotomy of future specimens can be coordinated to be as long as possible from the last infusion. This becomes difficult if the patient is receiving extended infusions (eg, parenteral nutrition; extended propofol infusion for sedation). If lipemia results from a medical condition, timing of phlebotomy may have minimal impact. There are some maneuvers to reduce lipemia in a specimen. The 2 primary approaches are addition of lipid-clearing reagents or ultracentrifugation followed by manual removal of the lipid layer. Either approach

has limitations. Lipid-clearing reagents do not require special instrumentation but may themselves interfere with various chemistry assays. Ultracentrifugation requires specialized equipment that may not be available in many laboratories; however, this approach does not involve adding additional reagents to the specimen. For the rare patients who have severe lipemia and subsequent in vitro hemolysis, alternative approaches can be tried such as performing analysis on whole blood in a blood gas analyzer quickly after obtaining specimen. This approach is limited in number of tests available but does minimize sample processing time.

Teaching Points

- Hemolysis and lipemia are 2 common preanalytical interferences that can impact laboratory testing. Hemolysis visually appears as pinkish or reddish plasma/serum. Lipemic plasma or serum appears turbid and opaque.
- The most common causes of hemolysis are associated with phlebotomy and specimen transport/processing (in vitro factors). Poor phlebotomy technique is the major overall cause. Hemolysis is less commonly due to medical factors such as hemolytic anemia or hemoglobinopathies (in vivo factors).
- The most common cause of lipemia is nonfasting, with recent ingestion of lipid-containing meal. More severe lipemia results from a disease condition causing hypertriglyceridemia (eg, diabetes, genetic hyperlipidemia) or recent intravenous infusion of a lipid emulsion.
- The main impact of hemolysis on laboratory tests are elevation of analytes found in high concentrations in erythrocytes (eg, aspartate aminotransferase, lactate dehydrogenase, potassium), decrease of peptides (eg, insulin) due to action of erythrocyte proteases, or interference with spectrophotometric readings.
- Lipemic interferences are more variable and usually evident only with severe lipemia. The presence of severe lipemia can interfere with spectrophotometric readings, volume sensing, and pipetting by automated instruments, and alter electrolyte readings by volume displacement.
- Hemolysis and lipemia can be detected visually, although this is more prone to inaccuracy and interindividual variability. Automated clinical chemistry instruments can detect hemolysis and lipemia by rapid spectrophotometric readings.
- Prevention and minimization of hemolysis mostly focuses on promoting optimal phlebotomy and specimen transport/processing. A common first step for a patient whose samples show hemolysis is to have someone else (ideally experienced with phlebotomy) perform the venipuncture.
- Lipemia due to a medical condition is difficult to prevent. When lipemia is related to intravenous lipid emulsion infusions, phlebotomy should ideally be performed as long as feasible after the infusion ends. Ultracentrifugation and lipid-clearing agents are maneuvers that can reduce lipids in a sample.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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