

LabNotes[†]

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A Newsletter
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The Hemolyzed Specimen: Causes, Effects, and Reduction

By Valerie Bush, PhD.
Lynn Mangan, MT, (ASCP)

The issue of hemolysis has always plagued clinical laboratories and continues to be a growing concern. In many hospitals, nurses and other healthcare workers have replaced traditional teams of highly skilled phlebotomists. Often this "decentralization", as it is called, occurs with little or no phlebotomy training for the new staff, as facilities make the flawed assumption that "sticking" patients to obtain blood is a simple procedure. In fact, a great deal of knowledge, skill, and experience is necessary to collect a quality blood specimen that yields the desired results.

The high degree of variability in the training, skills, and frequency of phlebotomy practice of the non-laboratory staff is a major factor in the increase of hemolysis rates in many facilities. Hemolysis, defined as red blood cell breakdown and the release of hemoglobin and intracellular contents into the plasma, is the major cause for specimen rejection as shown by the College of American Pathologists (CAP) Chemistry Specimen Acceptance Q-Probes study.¹ In fact, some facilities have gone back to the use of centralized phlebotomy teams in order to alleviate the quality issues associated with poor collection.

Hemolysis leads to a higher rate of rejected specimens and is a cause of frustration for both the lab and the "floors". More often than not, rejected samples and inaccurate results are attributed to alleged "laboratory errors," with the blame usually placed on the medical technologists. Rarely is a connection made between improperly collected specimens and inaccurate laboratory results.

Hemolysis can be recognized in the laboratory by a visual inspection of the plasma or serum sample, which

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LabNotes



From The Editor

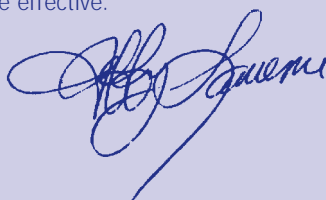
My professional career in pathology and laboratory medicine is endowed with many different perspectives — physician, academic pathologist, research pathologist, and today as Vice President of Medical and Scientific Affairs for Becton Dickinson. Change has been the one constant, as we have collectively worked to improve the accuracy of laboratory tests and to adjust performance goals and quality standards as technology has evolved. The end goal is always to take better care of our patients.

As we publish in medical and laboratory journals, you may hear from me directly from time to time. But my primary vocation as Editor of **LabNotes** is to guide the editorial focus of this newsletter so that we are as relevant to you, our friends and customers, as we can be.

In that vein, I would like to sincerely thank those of you who have taken the time to let us know what you are interested in reading about in **LabNotes**. Your input is an invaluable resource to us. Please continue to inform us of your information needs and concerns.

One of the areas that you suggested is the problem of hemolysis. Laboratories and hospitals are continuously working to find ways to make blood collection practices more efficient, to increase the accuracy of laboratory results, and to deliver the best patient care. In this issue of **LabNotes** we take a very practical look at hemolysis, its causes and effects and, most importantly, its reduction.

All of us must continually learn and adapt in a changing healthcare environment. I hope that **LabNotes** serves as a valuable resource to you as we work to make our healthcare workplace safer and more effective.



Jeffrey B. Lawrence, *M.D.*
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Associate Editor, Leslie S. Magee, *MBA, MT(ASCP)*
Editorial Contributor, Vera Bitcon, *MS, MT(ASCP)*
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Address all correspondence to: Leslie Magee, Associate Editor, LAB NOTES, BD Vacutainer Systems, *Preanalytical Solutions*, 1 Becton Drive MC325, Franklin Lakes, NJ 07417-1885.

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appears rosy to bright red in color. Results from all laboratory disciplines can be affected by hemolysis, especially in chemistry. Some of the more routine tests involved are: potassium, sodium, calcium, magnesium, bilirubin, haptoglobin, total protein, aldolase, amylase, LD, AST, ALT, phosphorus, alkaline phosphatase, acid phosphatase, GGT, folate, and iron.²

Specimen Collection Techniques

The major causes of hemolysis are improper specimen collection and handling. Therefore, proper training and education can significantly reduce the number of hemolyzed specimens received in the laboratory. The factors listed below are all known to cause hemolysis to varying degrees and should all be taken into consideration during collection.

Vein size and trauma—Puncturing small, fragile veins and probing or “fishing” the vein with a needle can lead to hemolysis. Choose an appropriately-sized vein and use phlebotomy equipment suitable for the vein size. If the vein is fragile, do not use large volume tubes. If a vein is traumatized during puncture, the first tube collected may be hemolyzed, while subsequent tubes are fine. Avoid puncturing areas that have a hematoma.

Alcohol preparation—Allow the alcohol to dry completely prior to venipuncture. The needle can transfer wet alcohol from the skin into the blood specimen and cause hemolysis.

Needle size—Using a large needle (larger bore=lower gauge) can cause hemolysis by allowing a large amount of blood to suddenly enter the tube with great force. Similarly, the use of needles that are too fine (higher

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gauge) can also cause hemolysis by forcing the blood through an extremely small opening under a great force. The red cell walls become sheared on the needle as they enter the tube.⁴

Loose connections—Ensure that all connections of the collection components are tightened, i.e., the connections between a blood collection set and luer adapter, between syringe and needle, and between catheter and luer adapter. Loose connections introduce air into the system and cause frothing in the specimen, which can result in hemolysis.

Underfilled Tubes—Fill all tubes to full capacity to ensure the proper blood-to-additive ratio. Certain additives in high concentrations, such as sodium fluoride, can cause varying degrees of hemolysis.⁵

Syringe Collections—Improper syringe draws are notorious for causing hemolyzed specimens. Syringe use should be avoided, if possible, in favor of the evacuated tube system. A study was conducted to evaluate the effects of specimen quality when using syringe draws, compared to the evacuated tube system. Visual hemolysis was found in 19% of specimens drawn by syringe, compared to 3% when drawn by the evacuated tube system. In addition, syringe-collected samples exhibited clotted EDTA specimens in 11% of the patients, as opposed to none in the evacuated tube system.⁶

If a syringe must be used, the following recommendations can reduce the incidence of hemolysis:

- Pump the plunger 2-3 times prior to collection to loosen the plunger.
- Tighten the needle and syringe connection.

- Use a 3-10mL syringe, avoiding larger volumes if possible.
- Ensure that the speed of aspiration does not exceed 1mL of air space during collection. Excessive aspiration forces frequently cause hemolysis.⁷
- Perform blood transfer into the tube immediately.
- Fill tube by vacuum only. NEVER push down on the plunger; this increases the force of the blood flow, creating a high degree of red blood cell (RBC) trauma. More importantly, positive pressure is produced in the tube, with a potential to cause either tube breakage or stoppers to pop out.
- Use a blood transfer device to transfer syringe-collected blood into a tube. It will enhance safety and improve specimen quality.
- Angle the syringe so that the blood runs down the side of the tube. By preventing the cells from hitting the

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bottom of the tube with such a great force, RBC trauma can be reduced.

Peripheral Catheter Collections—The highest rates of hemolyzed specimens appear to come from the acute care setting, i.e., Emergency Dept (ED), Labor and Delivery (L&D), and Intensive Care Units (ICU).³ Studies have shown that the main source of hemolysis in the ER is the use of IV catheters for specimen collection. One study found that specimens drawn by nurses through an IV catheter were more than 3 times as likely to be hemolyzed than those drawn by venipuncture (13.7% vs 3.8%).⁸ In another study conducted at the University of Virginia Health Sciences Center, specimens were collected using IV catheters and venipuncture, and then compared. The results were striking, revealing a 50% rate of hemolysis in the IV catheter collected specimens,

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compared to no hemolysis at all in the [peripheral] venipuncture specimens.⁹

Blood collected from the back of the IV catheter is pulled through several gauges; the catheter generally ranges from 18 to 22G, the Luer adapter “front” end is 15G, and the stopper-piercing needle is 20G. Slowing down this “pull rate” can reduce the hemolysis rate significantly. The use of partial draw collection tubes is an effective way to slow down the pressure exerted on the blood and, thus reduce hemolysis.¹⁰ The reduced vacuum in these tubes yields a slower, gentler draw. Partial draw

tubes are designed to fill “part way” while maintaining the proper blood-to-additive ratio. The smaller volume of blood drawn into partial draw tubes satisfies the CAP recommendation for minimizing large blood draw volumes (Checklist Question: 01:40500), and mitigates safety concerns as less blood is handled and discarded. (Note: Partial draw tubes should not be confused with small size pediatric tubes that are fully evacuated.)

Specimen Handling Techniques

Once the proper specimen collection techniques are applied, subsequent specimen handling factors must be considered to prevent hemolysis from occurring in the pre-analytical phase. Consider the factors listed below to prevent hemolysis:

Mixing Tubes—Mix the blood with the tube additive through gentle tube inversions. Do not shake the tube after collection.

Transport Methods—Be cautious with pneumatic tube systems and other rough transport conditions that can create turbulence and RBC trauma within the tube. Hand deliver specimens when feasible. Specimens should be stored in an upright position following centrifugation.

Rimming clots—Do not use wooden applicator sticks to rim clots, which can shred the red cells. With the current evacuated serum tubes available, rimming clots is unnecessary.

Temperature—Store and transport specimens in regulated temperature conditions, as temperatures that are

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too high or too low can rupture red cell membranes. Check to make sure that centrifuge temperatures are acceptable. Abide by the recommended transport and storage temperatures specified by the laboratory that is performing the assays.

Quality Specimens, Quality Test Results

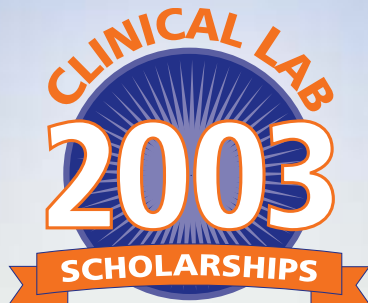
Proper specimen collection and handling techniques are critical to producing quality laboratory results. Although there will occasionally be sources of hemolysis that are out

of the control of the phlebotomist, the above recommendations, if followed, should greatly decrease the incidence of hemolyzed specimens in the laboratory.

Consistent quality specimens can only result from proper training and knowledge of the factors that can influence lab results. The bottom line is to obtain accurate test results that truly reflect the patient's status. To ensure that this happens, facilities should establish standard protocols for specimen collection, and make certain that the proper training and expertise are in place with each potential phlebotomist. ■

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Our Clinical Lab Scholarship program is one of the many ways that BD Vacutainer, *Preanalytical Solutions* supports the continuing education of medical professionals. Each year, scholarships are awarded to individuals from laboratories and hospitals across the United States.

Our upcoming scholarship program will grant each recipient a trip to the 2003 CLMA/ASCP Conference, to be held June 21-24, 2003 in Salt Lake City. Scholarship recipients are also invited to attend an **exclusive** dinner meeting with keynote addresses by prominent clinicians.

Visit our website at www.bd.com/vacutainer/scholarship for additional information on this exciting scholarship program and for information on when to enter.

Legislative Alert Needleless Blood Transfer

“... If drawing blood with a syringe is necessary, engineering controls (engineered sharps injury protection) and safe work practices (including mechanical means of removal if available) must be used and **needleless blood transfer devices must be implemented.**”

Occupational Safety & Health Administration (OSHA),
US Department of Labor

June 12, 2002 OSHA Standard Interpretation
letter on the Re-use of blood tube holders.



Reference
#364880

BD Vacutainer™
Blood Transfer Device

Understanding Additives: EDTA

Ethylenediaminetetraacetic acid (EDTA) salts are often used as anticoagulants for blood specimens, especially for hematology testing. The salts of EDTA are aminopolycarboxylic acids, which act as chelating agents. Chelation activity takes place by binding calcium, thus preventing clotting. EDTA also reacts with other divalent cations that can act as enzyme cofactors.

The three different salts of EDTA (disodium, dipotassium, and tripotassium) are currently used for specimen collection. The quantity of EDTA used in specimen tubes is based on the normal amount of calcium in plasma that can be complexed to the anticoagulant: 1.15 mmol/L. The amount of EDTA considered optimal is 1.8 mg EDTA per 1 mL of blood. It should be noted that the total amount of calcium in plasma is about 2.5 mmol/L, of which half is bound to proteins.

The International Council for Standardization in Haematology and NCCLS have recommended K₂EDTA as the anticoagulant of choice for blood cell counting and sizing for the following reasons:^{1,2}

- K₃EDTA results in greater RBC shrinkage with increasing EDTA concentrations (11% shrinkage with 7.5 mg/mL blood).
- K₃EDTA produces a larger increase in cell volume on standing (1.6% increase after 4 hours).
- K₃EDTA leads to lower MCV values (typically a -0.1 to -1.3% difference is observed compared with K₂EDTA).



- K₃EDTA is a liquid additive and, therefore, will result in the dilution of the specimen. All directly measured values (Hgb, RBC, WBC, and platelet counts) have been reported to be 1-2% lower than results obtained with K₂EDTA.^{2,3}

- With some instrument systems, K₃EDTA gives lower WBC counts when used at high concentrations. Brunson, et al., reported that plastic tubes containing K₂EDTA gave complete blood count and differential results in excellent agreement with glass tubes containing K₃EDTA, though they confirmed the earlier results of 1-2% higher WBC, RBC, hemoglobin, and platelet count results with the former tube, owing to dilution observed with K₃EDTA.⁴
- Our internal studies showed no clinically significant differences when comparing K₃EDTA glass tubes to K₂EDTA plastic tubes.^{5,6}

It is important that the proper amount of blood be drawn into EDTA tubes, with the caution to avoid short draws.

• • •

The International Council for Standardization in Haematology and NCCLS have recommended K₂EDTA as the anticoagulant of choice for blood cell counting and sizing.

EDTA is used as the anticoagulant of choice in blood collections for complete blood counts (CBC), microhematocrits (packed cell volume [PCV]), differential leukocyte counts, platelet counts, reticulocyte counts and flow

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NCCLS (National Committee for Clinical Laboratory Standards) is a globally recognized, voluntary consensus standards-developing organization that enhances the value of medical testing and healthcare services through the development and dissemination of standards, guidelines, and best practices. It is comprised of over 2000 member organizations worldwide from government, industry, and the professions.

NCCLS brings together representatives from every facet of clinical laboratory testing in an unbiased forum to solve problems. Three groups first organized it in 1968: laboratory professionals, the industries that supply and support them, and the regulatory and non-regulatory governmental agencies concerned with the clinical laboratory. All three groups realized the need to develop practical, clinically relevant standards.

The end result is usually a published standard or guideline, but the NCCLS forum might take other forms, such as a conference, a set of consensus recommendations, general information, an educational workshop, or a video.

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www.nccls.org

What's New at NCCLS?

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C30-A2 Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities; Approved Guideline

This document provides guidance for performing point-of-care blood glucose tests, with an emphasis on quality control, training, and administrative responsibility.

X3-R Implementing a Needlestick and Sharps Injury Prevention Program in the Clinical Laboratory; A Report

This report presents a step-by-step approach for implementing safer medical devices that reduce or eliminate sharps injuries to laboratory personnel.

GPO2-A4 Clinical Laboratory Technical Procedure Manuals; Approved Guideline – Fourth Edition

This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the laboratory testing community.

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Guidance on safe handling and disposal of chemical, infectious, radioactive, and physical waste generated in the clinical laboratory.

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MM2-A2 Immunoglobulin and T-Cell Receptor Gene Rearrangement Assays; Approved Guideline-Second Edition

This document provides guidance for conducting molecular tests of immunoglobulin and T-cell receptor gene arrangements.

MM6-P Quantitative Molecular Methods for Infectious Diseases; Proposed Guideline

This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It also presents recommendations for quality assurance, proficiency testing and interpretation of results.

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cytometry. It is not generally considered to be an acceptable anticoagulant for specimens for coagulation tests, except for isolation and examination of platelets. EDTA specimens are not suitable for calcium, iron, alkaline phosphatase, creatine kinase and leucine aminopeptidase determinations.

It is important that the proper amount of blood be drawn into EDTA tubes, with the caution to avoid short draws. An excess of EDTA in the blood may increase osmotic pressure and distort or rupture cells. Excess EDTA causes shrinkage of red cells with resulting decreases in hematocrit, mean corpuscular volume (MCV)

and red cell distribution width (RDW). Artifactual changes are also seen on morphology smears prepared from this type of specimen.

As soon as blood is collected in an EDTA tube, it should be inverted 8 to 10 times to ensure thorough mixing and proper anticoagulation of the specimen. ■

Our internal studies showed no clinically significant differences when comparing K₃EDTA glass tubes to K₂EDTA plastic tubes.

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