Troubleshooting hemolysis issues

Hemolysis continues to be a challenge and concern for the clinical laboratory. Recollection and subsequent patient treatment delays are costly to healthcare systems. Factors that contribute to red cell lysis are listed below with suggested corrective actions.

Specimen collection issues

Possible factors affecting hemolysis	Possible consequences	Corrective actions (follow your institution's policies)
• Venipuncture site • Drawing from a distal site to the antecubital region of the arm, has been shown to result in more hemolysis[1]	• Since hand veins are fragile, they can easily be traumatized resulting in hemolysis.	• Redraw the specimen. • The preferred venipuncture site is the antecubital fossa, where 3 major veins are located. Phlebotomists must prioritize the median cubital vein then cephalic vein; the basilic vein is the least preferred vein for performing a venipuncture. • If it is necessary to draw distally to the antecubital area, a 22 G or 23 G needle used with a partial draw tube (i.e. has a reduced vacuum and tube fills "part way" while maintaining the proper blood-to-additive ratio) is suggested.[2,8,9]
• Prolonged tourniquet time[2]	• Hemoconcentration. • May increase values for protein-based analytes, packed cell volume, and other cellular elements. • Affected analytes include but not limited to: albumin, calcium, potassium, red blood cells, white blood cells, differential, hemoglobin, hematocrit, glucose triglyceride, total protein, and alkaline phosphatase.[2]	• The tourniquet should be released after no more than one minute from initial placement, and excessive fist clenching should be avoided.[2]
• Needle probing or readjustment[2]	• Vein trauma may result when the needle placement is not accurate.[2]	• The needle should be parallel to the vein. Enter at a 30° angle or less.[2] • Reposition the needle forward or backward (vertically).[2] • Avoid probing.[2]
• Needle gauge[2] – Large bore (= lower gauge) Ex. 18 G – Small bore (= higher gauge) Ex. 25 G	• The use of a large-bore needle may result in a much faster and more forceful flow of blood through the needle, resulting in hemolysis.[3] • The use of a small-bore needle, resulting in a large vacuum force applied to the blood, may cause shear stress on the red blood cells, causing them to rupture.[2]	• Use a partial draw tube, if available. • The choice of the needle gauge size is dependent on the patient's physical characteristics, the venipuncture site and amount of blood to be drawn.[2] • Avoid using a needle with an internal diameter that is too small or too large.[2,3]
• Syringe collection[2]	• Pulling the plunger of a syringe back too far while using a large bore needle, may cause enough pressure for hemolysis to result during collection. The pressure may be greater than a standardized evacuated tube.[2] • Transferring into a tube by pushing down on the syringe plunger to force blood into a tube may cause hemolysis.[2]	• In general, venipuncture using a traditional needle and syringe should be avoided for safety reasons, however if conditions require the use of a syringe, the following is suggested:[2] - Before use, move the plunger within the barrel of the syringe to ensure freedom of movement.[2] - Avoid drawing the syringe plunger back too forcefully when collecting blood with a needle and syringe.[2] - Immediately after venipuncture, use a BD Vacutainer® Blood Transfer Device when transferring blood from the syringe into the tube without pushing the plunger.[2] - After attachment to the BD Vacutainer® Blood Transfer Device, angle the syringe. This allows the blood to flow slowly down the side of the tube wall.[4]
• Catheter collection[5]	• Several studies have noted that when blood is drawn from a peripheral IV catheter, a higher incidence of hemolysis could occur due to changes in the internal diameters (catheter and connectors) which may cause turbulence and result in cell disruption.[6,7]	• Ensure all connections fit together securely. • Ensure compatibility among all components of devices used.[2] • Collect discard tube. • Use partial draw tubes.[8,9] • Use extension tubing if available. • Use the BD Vacutainer® Luer-Lok™ Access Device (LLAD) for the collection of blood from catheter ports.[10]
• Loose connections of component parts (i.e., needle to holder, blood collection set hub to luer adapter or syringe)[2]	• Air may be introduced into the sample and cause frothing. This may result in hemolysis.[2]	• Ensure all connections fit together securely. • Ensure compatibility among all components of devices used.[2]
• Tube choice[2]	• The vacuum pressure of large full draw tubes may cause the blood to enter the tube forcefully and may cause cell rupture.	• Based upon the condition of the patient's vein, select a tube with the appropriate amount of volume.[2] • The use of partial draw collection tubes is an effective way to slow down the pressure exerted on the blood. The reduced vacuum in these tubes yields a slower, gentler draw. Partial draw tubes are designed to fill only partially, while retaining the standard external configuration, thereby maintaining instrument compatibility.[9]
• Tube fill volume (under filling)	• Excessive concentrations of additives can cause rupture of the RBC cell membrane, e.g., oxalate.[11,13]	• Fill tubes to their proper draw volume to ensure proper blood:additive ratio.[12]
• Hematoma[13]	• Specimens collected by penetrating through a hematoma may cause erroneous test results.[13]	• Select another site.[2]

Processing/handling/transport issues

Possible factors affecting hemolysis	Possible consequences	Corrective actions (follow your institution's policies)
• Vigorous mixing	• May cause RBCs to rupture.[7]	• Gentle tube inversion, according to the instructions for use.[7,12]
• Prolonged contact of serum/plasma with cells	• Hemoglobin released from hemolyzed cells will contaminate serum/plasma and may cause errors to test results.[13]	• Separation of serum or plasma from the cells should take place within 2 hours of collection to prevent erroneous test results unless conclusive evidence indicates that longer contact times do not contribute to result error.[7,12]
• Centrifugation at excessive g-force[14] • Increased heat exposure in centrifuge[14,15]	• Causes lysis of cells. • Stability of tests may be impacted by heat generated in the centrifuge chamber.[15]	• Ensure centrifuge temperatures and settings are acceptable: – Open lid to allow inner chamber to cool. • Follow manufacturer recommendations for centrifugation conditions.[12] • Refer to centrifuge manufacturers operators manual.
• Temperatures[7] – Elevated or Decreased	• RBC membrane may rupture[7]	• Keep specimens at room temperature.[7] • Chill specimens, if necessary, avoid freezing, unless recommended.[7] • Protect specimen from extreme cold or heat.[7]
• Pneumatic tube system[16]	• Hemolysis may be due to acceleration and/or deceleration speed, length of system, angles, and tube cushioning.[16,17]	• Hand-deliver specimens when possible. • Ensure the pneumatic tube system has been tested to certify test results will not be compromised.[7] • Provide adequate inner cushioning for the blood collection tubes during transport to avoid excessive mixing.[16,17]

Patient factors

Possible factors affecting hemolysis	Possible consequences	Corrective actions (follow your institution's policies)
• Metabolic disorders[18,19] – Liver disease – Sickle cell anemia – Autoimmune hemolytic anemia	May cause red cell lysis.[18]	• Check patient's history.
• Chemical agents[18,19] – Lead – Sulfonamides – Antimalarial drugs – Analgesics	• Depending on the dosage, may cause red cell lysis.[18]	• Check patient's history.
• Physical agents[18,19] – Mechanical heart valve – Third degree burns	• May cause intravascular hemolysis. • Cause direct damage to the RBCs.[18]	• Check patient's history.
• Infectious agents[18,19] – Parasites – Bacteria	• Fragility of RBCs may increase.[18]	• Check patient's history.

References: **1.** Burns ER, Yoshikawa N. Hemolysis in serum samples drawn by emergency department personnel versus laboratory phlebotomists. *Lab Med.* 2002; 33: 378 –380. **2.** CLSI. *Collection of Diagnostic Venous Blood Specimens. 7th ed.* CLSI standard GP41. Wayne, PA: Clinical and Laboratory Standards Institute; 2017. **3.** Sharp MK, Mohammad SF. Scaling of hemolysis in needles and catheters. *Annals of Biomedical Engineering.* 1998;26(5):788-797. doi:10.1114/1.65 **4.** Stankovic AK, Smith S. Elevated serum potassium values: the role of preanalytical variables. *Am J Clin Pathol.* 2004;121(suppl 1): S105-S112 **5.** 1Kennedy C, Angemuller S, King R, et al. A comparison of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. *J Emer Nurs* 1996; 22: 566-9. **6.** Wollowitz A, Bijur PE, Esses D, John Gallagher E, Jang TB. Use of Butterfly Needles to Draw Blood Is Independently Associated With Marked Reduction in Hemolysis Compared to Intravenous Catheter. *Acad Emerg Med.* 2013;20(11):1151-1155. doi:10.1111/acem.12245 **7.**CLSI. *Handling, Transport, Processing, and Storage of Blood Specimens for Routine Laboratory Examinations. 1st ed.* CLSI guideline PRE04. Clinical and Laboratory Standards Institute; 2023. **8.** Heyer NJ, Derzon JH, Winges L, et al. Effectiveness of practices to reduce blood sample hemolysis in EDs: a laboratory medicine best practices systematic review and meta-analysis. *Clin Biochem.* 2012;45(13–14):1012–103. **9.** Heiligers-Duckers, Peters, van Dijck, Hoeijmakers, Janssen. Low vacuum and discard tubes reduce hemolysis in samples drawn from intravenous catheters. *Clin Biochem.* 2013;46(12):1142-1144. doi:10.1016/j.clinbiochem.2013.04.005. **10.** IFU VDP40491 BD Vacutainer® Luer-Lok™ Access Device Holder with Pre-Attached Multiple Sample Adapter Instructions for Use at eifu.bd.com. **11.** Chan A, Chung H, Cockram C and Swaminathan R, Handling of Blood Specimens for Glucose Analysis, *J. Clin. Chem. Clin. Biochem* 1990;28:185-186. **12.** VDP40161(12) 2023-06 BD Vacutainer® Evacuated blood collection system Instructions for Use at eifu.bd.com. **13.** Ana-Maria Simundic, Geoffrey Baird, Janne Cadamuro, Seán J. Costelloe & Giuseppe Lippi (2019): Managing hemolyzed samples in clinical laboratories, *Critical Reviews in Clinical Laboratory Sciences*, DOI: 10.1080/10408363.2019.1664391 **14**. S. Charantimath V, S. Bubanale V. Effect of centrifugation force and time on the analysis of lactate dehydrogenase and potassium in the serum samples. Biomedicine (Trivandrum). 2022;42(5):1094-1097. doi:10.51248/.v42i5.1915. **15.** Sample Temperature during High Throughput Ambient Temperature Centrifugation using Sigma 4-5L. (n.d.). https://www.sigma-zentrifugen.de/fileadmin/user_upload/sigma/produkte/pdf/White_paper__4-5L-Temperature_during_high_throughput_centrifugation.pdf. **16.** Kapoula GV, Kontou PI, Bagos PG. The impact of pneumatic tube system on routine laboratory parameters: a systematic review and meta-analysis. Clin Chem Lab Med. 2017;55(12):1834-1844. doi:10.1515/cclm-2017-0008. **17**. Pierre CC, Wiencek JR. The impact of environmental factors on external and internal specimen transport. *Clin Biochem.* 2023 May;115:13-21. doi: 10.1016/j.clinbiochem.2022.11.005. Epub 2022 Nov 13. PMID: 36379239. **18.** Wan Azman WN, Omar J, Koon TS, Tuan Ismail TS. Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories. *Oman Med J.* 2019;34(2):94-98. doi:10.5001/omj.2019.19. **19.** Karcher DS, McPherson RA. *Henry's Clinical Diagnosis and Management by Laboratory Methods; 18th Ed;* 1991. pgs. 642-669.

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