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What does the haemolysed sample mean for reliability of laboratory test results?

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ABSTRACT

Laboratory testing is an integral part of the decision-making process and laboratory results often strongly influence medical diagnosis and therapies. Laboratory data are a major aid to clinicians and therefore must be trustworthy, which means that errors should be kept to a minimum to ensure reliable laboratory data. Haemolysed samples frequently occur in clinical laboratories. If they are not identified and managed adequately, there is a possibility of reporting erroneous results to clinicians. This article focuses on this issue, providing an overview of impact of haemolysis on laboratory test results and of the manner in which the laboratory deals with haemolysed samples. We show the influence of haemolysis on high-sensitive troponin T measurements. We conclude that the use of new technology, which can aid in detecting haemolysis, and the education of healthcare personnel, which can improve the pre-analytical phase of the laboratory process, can contribute to decreasing the number of errors and their unwanted impact on laboratory test results.

Key words: haemolysis, laboratory error, haemolysis index (HI), troponin T

In recent years, laboratory diagnostics has extended its effect not only on the diagnosis of pathologies, but also on identifying risk factors and monitoring treatments. Laboratory testing has therefore become an integral part of the decision-making process. This fact indicates that laboratory data are a major aid to clinicians and therefore must be trustworthy. It is thus essential that errors be kept to a minimum to ensure reliable laboratory data.

The definition of laboratory errors, recently acknowledged by the International Organization for Standardization (ISO), is as follows: "...any defect from ordering tests to reporting results and appropriately interpreting and reacting on these". (1) Errors reduce patient safety or otherwise contribute to adverse health outcomes for the quality of patient health care and patient satisfaction.

The complete testing process begins with the physicianžs formulation of the clinical hypothesis and the selection of the most appropriate examinations. This is followed by three phases:

the pre-analytical process, which includes patient preparation, sampling and handling of specimens,

the analytical process, and

the post-analytical process - result reporting to physicians.

Reliable laboratory results depend on the quality of each of these three phases.

Advances in technology (analytical techniques, automation, computer science) have greatly contributed to limiting the probability of errors and their adverse clinical outcome in the analytical phase of testing, but a large degree of variability still occurs in extra-analytical phases of the total testing process – especially in the pre-analytical setting which is incidentally one of the most labour-intensive activities. (2)

The most common pre-analytical problems are traditionally identified as inadequate collection procedures, including inappropriate quality of the specimen (haemolysis, clotting, contamination), insufficient volume, inappropriate containers and misidentification. Haemolysed blood samples are frequently received in clinical laboratories, comprising as much as 3.3% of all routine samples and accounting for up to 40%–70% of all unsuitable samples identified — nearly five times higher than other causes, such as insufficient, incorrect, and clotted samples. (3) *In vitro* haemolysis remains the leading cause of unsuitable specimens for both outpatient and inpatient samples, for routine and STAT specimens. (4, 5) However, several studies identified haemolysis rates that were significantly elevated in the emergency department compared to the rates in other hospital departments. (6)

Haemolysis

Haemolysis, from the Latin *haemo* (blood) and *lysis* (to break open), is the release of haemoglobin and other intracellular components from blood cells to the surrounding plasma following the damage or disruption of the cell membrane. Haemolysis is generally a pre-analytical problem that can be avoided. Haemolysis due to the break-

down of blood cells is important to the laboratory because it can have an effect on laboratory results. Haemolysis can occur *in vitro* and *in vivo*. Haemolysis *in vitro* occurs at the time of specimen collection, transportation or centrifugation. The skill in specimen collection is the most important factor which is often neglected. (1) Haemolysis can also occur *in vivo* (in a patient) due to a variety of medical conditions, including antigen-antibody reactions, haemolytic anaemias, toxins and poisons, mechanical RBC rupture due to artificial heart valves, as well as treatments such as haemodialysis and the use of the heart-lung bypass machine.

Visually, haemolysis is determined on the basis of free haemoglobin concentrations above 300 mg/L which confers a detectable pink to red hue to serum or plasma. (7)

Haemolysis is graded according to the visible presence of haemoglobin as slight, moderate and marked:

Slight haemolysis has little effect on most test values and the samples are usually processed.

Moderate haemolysis has some effect on some test results.

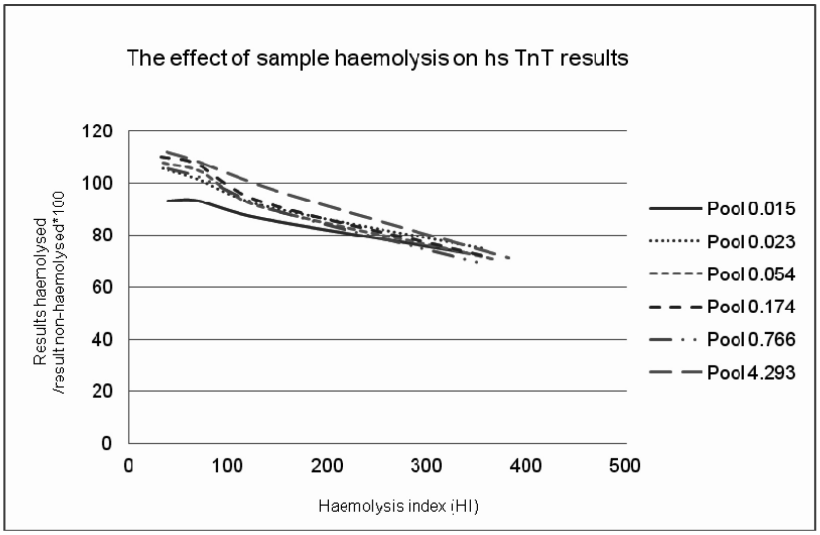
Marked haemolysis can have considerable effect on test results and samples should generally be rejected. It causes a slight dilution of analytes present at a lower concentration in the red cells compared to their concentration in plasma, and marked elevation of analytes when they are present at a higher concentration in red cells than in plasma.

Haemolysed samples can produce unreliable laboratory results and the effects can be the result of products released from the blood cells themselves or due to spectral interferences with laboratory analyzers.

When haemolysis occurs, the blood is unsuitable for many laboratory analyses (e.g. potassium, lactate dehydrogenase, aspartate aminotransferase, coagulation testing). (8) The cellular components of blood cells as a contaminant may, in fact, raise concentration of an analyte, given that the intracellular concentration of lactate dehydrogenase is 160-fold greater, potassium 22-fold greater, and magnesium 3-fold greater than in the plasma. This increases the concentration of lactate dehydrogenase by 670 U/L, potassium by 3 mmol/L, and serum magnesium concentration by 0.05 mmol/L per each 1000 mg/L of haemoglobin. (9-10) Haemoglobin at a concentration of 5 g/L (marked haemolysis) inhibits serum lipase activity by 50%. (11) However, these elevations in analytes are false only if haemolysis occurs *in vitro*.

Due to interferences with laboratory analysers, the degree of haemolysis influence depends on the methodology and individual instrumentation.

In vitro haemolysis has traditionally been assessed arbitrarily through visual inspection by laboratory personnel. More recently, several pre-analytical modules and analytical platforms have been equipped with systems capable of automatically testing a broad series of analytical interferences, including haemolysis. In most cases, the instruments report a qualitative or semi-quantitative žžhaemolysis index'' (HI) which should be compared with manufacturer-specific, instrument-specific and analyte-specific alert values before deciding whether to perform the analysis or report on the test results. The user can often adjust the level at which the interference generates an alert. Using this system, visual inspection is avoided and this improves the recognition of specimens with mild haemolysis (600 mg/L of free haemoglobin), which are difficult to detect by visual inspection, but might still be unsuitable for the measurements of several analytes such as aspartate aminotransferase, lactate dehydrogenase and potassium. (12)



hs TnT, high sensitive troponin T

Figure 1. The effect of sample haemolysis on hs TnT results

The influence of haemolysis on high sensitive troponin T (hs TnT) measurement

The high-sensitive troponin T (hs TnT) is the biochemical marker of choice for early diagnosis of acute coronary syndrome (ACS) and for the follow-up of dynamic changes in hs TnT concentration, especially in an emergency department where it is necessary to obtain results as soon as possible. Specimen haemolysis can cause significant interference in the measurement of hs TnT (13,14) and could prevent sample analysis which prolongs the turn-around time (TAT) and could potentially be very harmful for critical patients. This is why repeated blood sampling should be avoided whenever possible. In a STAT laboratory we quantify cell-free haemoglobin in plasma or serum by absorbance measurements

on a biochemistry analyser. The concentration of the cell-free haemoglobin is reported as the "haemolysis index" (HI). Each reagent manufacturer specifies the HI at which the analysis is permitted. As part of our laboratory method verification we evaluated the degree of haemolysis that affects the hs TnT assay. We prepared six lithium-heparin plasma pool samples that contained concentrations of 0.015, 0.024, 0.057, 0.189, 0.796 and 4.290 μg/L hs TnT. We added haemolysate to one aliquot of each pool to get four different haemoglobin concentrations of approximately 350, 700, 1500 and 3500 mg/L (HI: 35, 70, 150 and 350 respectively). The manufacturer specified that the assay is unaffected by haemolysis $<$1000 mg/L (HI $<$100). Our investigation showed similar results. The significant negative bias in the measurement of cTnT was observed as the concentration of haemoglobin increases (figure 1). It showed that HI can be increased to 130, thus additionally decreasing the need for repeated blood sampling and getting hs TnT result faster.

Conclusion

The use of new technology for detection and measurement of haemolysis can aid in detecting improper samples received in the laboratory and can decrease errors and their unwanted impact on the results of laboratory tests, thus minimizing the number of erroneous clinical decisions based on laboratory test results. Also important is the need to change blood collection practice in accordance with the guidelines for collecting samples. Additionally, the monitoring of the rejected samples and the identification of factors that caused sample rejection can contribute to avoiding errors and to promoting continuous quality improvements of laboratory service.

REFERENCES

1. ISO/WD TS 22367: Medical laboratories – Reduction of error through risk management and continual improvement.
2. Lippi G, Guidi GC. Risk management in the preanalytical phase of laboratory testing. Clin Chem Lab Med 2007;45:720–7.
3. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008;46:764-72.
4. Jones BA, Calam RR, Howanitz PJ. Chemistry specimen acceptability. A College of American Pathologists QProbes study of 453 laboratories. Arch Pathol Lab Med 1997;121:19–26.
5. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem 2007;53:1338–42.
6. Pretlow L, Gandy T, Leibach EK, Russell B, Kraj B. A quality improvement cycle: hemolyzed specimens in the emergency department. Clin Lab Sci 2008;21:219-24.
7. Thomas L. Haemolysis as influence and interference factor. eJIFCC vol 13(4). Available at: http://www.ifcc.org/ejifcc/vol13no4/130401002.htm.
8. Carraro P, Servidio G, Plebani M. Hemolyzed specimens: 2. a reason for rejection or a clinical challenge? Clin Chem 2000; 46: 306-7.
9. Brydon NG, Robert LB. The effect of hamolysis on the determination of plasma constituents. Clin Chim Acta 1972;41:435-8.
10. Elin RJ, Hosseini JM. Magnesium content of mononuclear blood cells. Clin Chem 1985;31:377-80.
11. Henry RJ, Sobel C, Berkman S. On the determination of "pancreatic lipase" in serum. Clin Chem 1957;3:77-89.
12. Ji JZ, Meng QH. Evaluation of the interference of hemoglobin, bilirubin and lipids on Roche Cobas 6000 assays. Clin Chim Acta 2011;412:1550-3.
13. Roche Diagnostics Technical Update. Notice of haemolysis effect on troponin T. 2003 (May).
14. Hammett-Stabler CA, Snyder JA, Chapman JF, Rogers MW, King MS, Phillips JC. Haemolysis interferes with troponin I and troponin T immunoassays. Clin Chem 2003;49:A89.