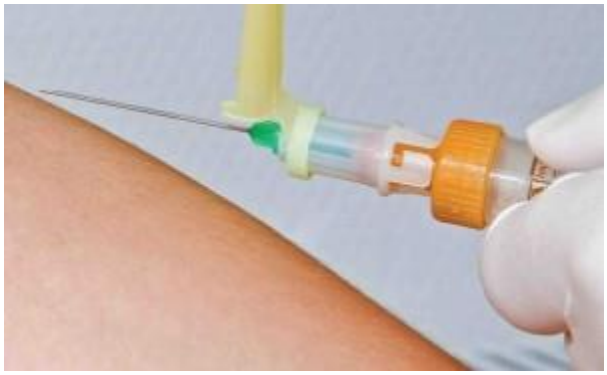
Haemolysis: a major challenge in the lab



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Haemolysis is defined as the release of haemoglobin and other intracellular components from erythrocytes, thrombocytes and leukocytes into the extracellular fluid i.e. the plasma or serum, following damage or disruption of cell membranes. Haemolysed specimen is the most common reason for rejection of specimens in the lab. Studies have shown that out of the total specimens received in the lab, 3.3% are found to be haemolysed, and out of the total rejected; 40-70% has been identified to be due to haemolysis. The American Society of Clinical Pathology has established a 2%, or lower, benchmark for haemolysis rates amongst laboratory blood samples.

Haemolysis may occur either *in vivo* or *in vitro*, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing. It has been observed that 3.2% of all detectable haemolysis is *in vivo. In vivo* haemolysis can originate from hereditary, acquired and iatrogenic conditions, such as autoimmune haemolytic anaemia, severe infections, intravascular disseminated coagulation, haemoglobinopathies, drug transfusion reactions etc. *In vivo*haemolysis does not depend on the techniques of the healthcare provider and is virtually unavoidable and cannot be resolved. *In vitro* haemolysis occurs more often and is caused by improper sample drawing, improper sample processing and improper sample transport (*see figure 1*).

▼FIGURE 1: *In vitro* haemolysis

IMPROPER SAMPLE DRAWING
Improper selection of the venipuncture site
Prolonged tourniquet time
Continuous clenching of the fist
Improper drying of the alcohol at venipuncture site
Needle gauge
Tube under filling
Syringe transfer
Catheter IV collection
Traumatic draw
Capillary collection

SPECIMEN PROCESSING
Excessive mixing or vigorous shaking of the tube
Not allowing clotting for sufficient time
Applicator sticks
Time delay before centrifugation
Centrifuge condition, speed, time and temperature

TRANSPORT
Mechanical trauma during transport
Temperature, humidity at the time of transport
Length, speed and number of times the sample is transported

Figure 1

IMPROPER SAMPLE DRAWING

One of the most important reasons for haemolysis is improper selection of the venipuncture site. Drawing blood from distal arm rather than anticubital fossa can result in haemolysis. A study done by Edward et al clearly shows that the anticubital fossa may be more favourable than the distal arm because of the faster flow of blood due increased diameter of the vein and reduced resistance. Difficulty to locate easy venous access, small or fragile veins (alternate sites to the anticubital area, such as small hand veins, are fragile and easily traumatised) unsatisfactory attempts, and missing veins can all lead to haemolysis. An improper venipuncture is indicated by a slow blood flow due to the lumen of the needle not centred in the vein, meaning the bevel opening of the needle is partly in the vein and partly in the vein wall, leading to an increasing aspiration force and rupture of the RBC.

The Clinical and Laboratory Standards Institute (CLSI) recommends the use of a tourniquet for localising suitable veins for ≤60 sec. Prolonged tourniquet time, or continuous clenching the fist, results in rupture of the RBC. Venous stasis greater than one minute affects potassium, calcium and albumin by 2.8%, 1.6% and 3.5% respectively, and venous stasis greater than three minutes affects potassium, calcium and albumin by 4.8%, 3.6% and 8.6% respectively.

Cleaning the vein puncture site with alcohol and not allowing the site to air dry results in transfer of alcohol from the skin to the blood specimen resulting in haemolysis. The choice of needle gauge size is very important. The use of large bore needles may result in a much faster and more forceful flow of blood through the needles resulting in haemolysis. Using a smaller gauge needle results in a large vacuum force applied to the blood and may cause sheer stress on the RBC, causing them to rupture. The ideal gauge of needle for venipuncture is usually 20-22. Haemolysis from excessive aspiration force is relatively frequent, mainly in cases of small or superficial veins. Pulling the syringe plunger back too fast or forcefully can result in haemolysis. Forcefully expelling the blood from the syringe into the blood tubes can also rupture the RBC.

A study done by Edward et al shows that under-filling tubes with blood will cause improper blood-to-additive ratios (especially EDTA), resulting in haemolysis. Haemolysis can be avoided by filling the tubes to the required level. When blood is drawn from a peripheral IV catheter, a higher incidence of haemolysis occurs due to frothing of the blood from a loose connection of the blood collection assemblies.

SPECIMEN PROCESSING

Excessive mixing or shaking of the blood after collection, not allowing to clot for recommended amount of time (30mins), use of applicator sticks to dislodge the fibrin, prolonged contact of serum or plasma with cells, failure to separate serum from red cells within 60 minutes of venipuncture, exposure to excessive heat or cold, can all cause RBC rupture and haemolysis. Centrifugation at too high a speed frequently compromises the integrity of the blood samples. Re-centrifugation (re-spin) of tubes with gel separators (the gel barrier may open and allow any supernatant that has been in contact with erythrocytes, to mix the supernatant previously above the separator).

TRANSPORTATION

Mechanical trauma during transport may occur, resulting in haemolysis. Variable factors such as temperature, humidity, length, speed, and number of times the specimen is transported, as well as the number of angles or turns the system uses also affects the integrity of the sample. Placing ice or frozen gel packs directly on tubes of blood can result in haemolysis.

HAEMOLYSIS INDEX/SERUM INDEX

The index aids in evaluating sample integrity by determining the level of haemoglobin in serum or plasma. It improves the quality of reported results, in almost no time, with minimal cost and also improved handling of paediatric samples.

The extent of haemolysis can be detected in two ways:

1. Visual haemolysis detection
2. Automated Serum Index.

Visual haemolysis detection Visually, haemolysis is defined as free haemoglobin concentration >30-50mg/dL conferring detectable pink/red hue to serum or plasma. It becomes clearly visible in specimens containing as low as 0.5% lysed erythrocytes. It is based on comparing patient samples with photographs of samples containing various concentrations of haemoglobin. Drawbacks of the visualising method are that it is unreliable since it may over-, and underestimate the actual prevalence of haemolysed serum specimens (i.e., trained observers are unable to accurately rank the degree of interference in serum). Elevated concentration of bilirubin may further impair the ability to detect haemolysis by visual inspection and therefore lead to serious underestimation of haemolysis in neonatal samples, where elevated bilirubin concentration is commonplace.

Automated Serum Index This is by directly measuring haemoglobin concentration photometrically followed by comparing it with the serum index values for haemolysis that have been determined by the instrument vendor. (These values represent the levels at which the haemoglobin significantly interferes with the analyte testing.) The advantage of an Automated Serum Index is its consistency, reproducibility and improvement in detection of haemolysis.

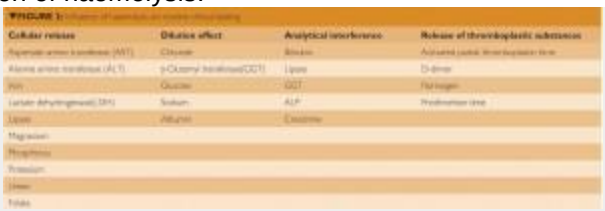

Figure 2

INFLUENCE OF HAEMOLYSIS ON ROUTINE CLINICAL TESTING

Haemolysis can influence routine clinical testing in the following ways (*see figure 2*):

- Leakage of haemoglobin and other intracellular components into the surrounding fluid may induces false elevation of some analytes or dilution effect. If the analyte in question is present in a higher concentration in blood cells than in plasma, then the analytical value will be increased. Conversely, if the concentration in blood cells is lower, the plasma becomes diluted, and the analytical result is too low. Caraway reported that erythrocytes contain 160-fold as much lactate dehydrogenase, 68-fold as much acid phosphatase, 40-fold as much aspartate aminotransferase, and 6.7-fold as much alanine aminotransferase as does plasma

- Haemoglobin absorbs light strongly at 415, 540 and 570nm. Haemolysis therefore increases absorption in this wavelength range and causes apparent increase in the concentration of analytes measured in this range
- In addition to haemoglobin, erythrocytes also contain proteins, enzymes, lipids and carbohydrates and many of these may also interact or compete with the assay reagents. E.g. free haemoglobin with its pseudo-peroxidase activity interferes in the bilirubin procedure by inhibiting the diazonium colour formation and thereby resulting in low values. Increased concentration of CK is most likely due to analytical interference, due to release of intracellular adenylate kinase, which is not completely inhibited under operating conditions
- Release of intracellular and thromboplastic substances from either leukocytes or platelets, results in prolongations in prothrombin time and dimerised plasmin fragments D(D-dimer) whereas shortening of activated partial thromboplastin time and decrease in fibrinogen values.

OVERCOMING THE CHALLENGE OF HAEMOLYSIS

There should be proper laboratory guidelines and recommendations for the management of haemolysed samples. Proper training and knowledge of the factors that can influence laboratory results, along with appropriate training of the phlebotomists, are essential prerequisites to minimise errors.

Every laboratory personnel should be trained properly. Standardised blood collection and handling procedures should be followed. Collection from a haematoma site and prolonged tourniquet time should be avoided. Equipment and connections that may lead to turbulent blood flow leading to haemolysis should be avoided. Vigorous mixing of the specimens after collection should be prevented and appropriate conditions of temperature and humidity should be maintained. Standardised practices for sample transportation and storage should be observed. The blood specimens should be centrifuged within a suitable time of collection, with appropriate conditions of centrifugation (force, spin, time and temperature), supernatant (serum or plasma) timely separated from the blood cells unless the primary tube is provided with a gel separator.

There has always been a debate as to whether we should or should not process the haemolysed samples. Basically when a haemolysed sample reaches the laboratory, we can; **1.** Reject the sample for analysis and ask for re-collection **2.**Perform the analysis and report the results with a comment **3.** When a haemolysed sample is referred to the laboratory, the personnel should always ask for new sample(s). In case new sample(s) cannot be obtained, it is the responsibility of the laboratory specialist to communicate the problem to the concerned physician and seek for the best solution for the best of the patient care.
It is pointed out by the authors of the recommendations that it is always better not to report the result rather than producing spurious data on unsuitable samples.

REFERENCES

References on request (magazine@informa.com)