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Preventing

Haemolysis

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Definition

Haemolysis

Is defined as the release of intracellular components of erythrocytes and other blood cells into the extracellular space of blood.

It is visible in non-icteric samples as a red colouration of serum or plasma. It is visible to the eye if the concentration is above 300mg/L.

The upper reference limit for free haemoglobin in plasma is 20mg/L; for serum it is 50 mg/L.

0.1% to 1% intact cells do not contribute to a haemolysis effect.

In vivo haemolysis *(3-4% of cases) is caused by antibodies (transfusion reaction), biochemically (through medication), by toxic substances, through hereditary factors (haemoglobulinopathies), through enzyme defects (acholuric jaundice) or by infections (malaria parasite infection affecting the invaded erythrocytes)*

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Definition

Differentiation of in vivo- and in vitro-haemolysis

Even if in vitro-haemolysis occurs more frequently, in vivo-haemolysis is of greater clinical importance because of its pathological origin.

In vivo released haemoglobin is bound to haptoglobin and transported into the reticuloendothelial system (RES), mostly to the spleen.

Free haemoglobin can only be measured in plasma if the haptoglobin transport capacity is exceeded. Consequently, haptoglobin is reduced.

The measurement of reduced concentration of haptoglobin thus permits an imperative assessment of haemolysis
(exceptions are inborn haptoglobin deficiency and newborn children).

Likewise the measurement of haemopexin and/or methaemoglobin / albumin was described for characterisation of in vivo haemolysis. In addition a rise in concentration of indirect bilirubin and reticulocytes is a typical sign of in vivo haemolysis leading in turn to increased erythropoiesis.

For financial reasons, usually samples are analysed for haemolysis using an automated spectrophotometer as well as visual inspection.

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Definition

Different types of haemolysis

1. *In vitro-haemolysis parallel increase of haemoglobin (red colouration of serum/plasma), K, LDH and AST respectively, but haptoglobin and reticulocyte-index remains normal.*
2. *Unforeseen increase in K, but no red colouration of serum/plasma, LDH in reference range, i.e. if whole blood is stored for several days.*
3. *In vivo-haemolysis parallel increase in haemoglobin (red colouration of serum/plasma), LDH but no parallel increase in K,*
4. *No colouration of s/p, but decrease of haptoglobin and potential increase in LDH, indirect bilirubin and/or reticulocyte-index, respectively.*
5. *Serum/plasma without red colouration, but increase in LDH, K, and acid phosphatase. In plasma no increase of these parameters (i.e. has been noticed in thrombocytosis.*

Effects

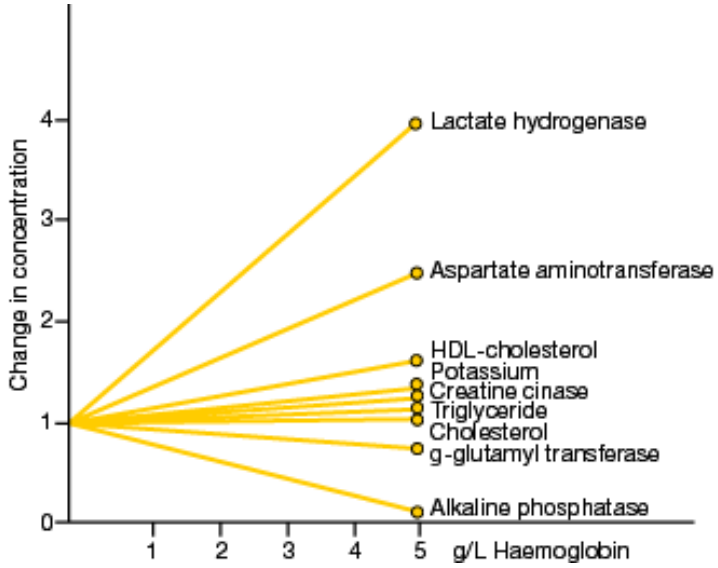
Effect of haemolysis on analysis

1. *The release of components from cells changes the concentrations in serum or plasma.*

   - concentration gradient between the cells and plasma (activity of AST in RBC 40 x higher)

2. *The red discolouration due to haemoglobin interferes with the photometric measuring.*

   - Haemoglobin absorbs light very strongly at 415 nm. Haemolysis therefore increases absorption in this wavelength and causes an apparent increase in the concentration of analytes measured in this range.



Changes of different parameters during haemolysis (Hb 5 g/L)

Effects

Effect of haemolysis on analysis

3. *Serum protein electrophoresis*

   Haemoglobin / haptoglobin complexes move between the alpha and beta globulin fractions. Free haemoglobin migrates as a diffuse reddish band in the beta globulin fraction.

4. *Chemical reactions during analysis can be influenced by cell substances.*

   Haemoglobin changes the molar extinction coefficient of the substrate or reaction product to be measured

Preventing Haemolysis

- **Physical stress before blood collection (stairs, morning jog)**
- *Allow outpatient to rest before blood collection (min. 5 minutes)*
- *No exhausting physical activity 3 days prior to giving a blood sample*
- *Take the sample from the patient sitting or lying down*

- **Excessive clenching of fist / hard tapping of vein**
- *Warm the puncture site (warm arm bath, a heating pad) for better visualisation of vein*

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Preventing Haemolysis

- **Tourniquet applied too long or too tightly**
- *Not longer than 1 minute, necessary to feel pulse – interstitial fluid may leak into the tissue*

- **Disinfection of puncture site**
- *Allow the disinfectant to dry according to instructions – otherwise disinfectant could get into the blood sample and corrupt the analysis results*

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Preventing Haemolysis

- **Excessive probing**
- *Choose an appropriately sized vein. Do not probe tissue to locate vein – this can lead to contamination due to tissue thromboplastin*
- *Avoid puncturing areas that have a haematoma*

- **Accessories**
- *Use of a too thin / thick needle is not recommended – could cause too high stress on cells*
- *Ensure that all connections of accessories are fitted together firmly – frothing!*
- *The highest rates of haemolysed specimens are reported from intensive care and emergency dep. – main source is the use of IV catheters*
  - *remains of infusion solution may contaminate the sample.*
  - *possibility of sharp edges on low quality catheters*

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Preventing Haemolysis

- **Filling of VACUETTE tubes**
- *Release the tourniquet on successful venipuncture*
- *Follow the recommended order of draw*
- *Fill the tubes correctly; Pay attention to mixing ratios*
- *Under filling of tubes causes high concentration of additives – NaF!*
- *Use paediatric draw volumes for fragile veins*
- *First tube collected may be haemolytic – use a discard tube*
- *When syringe is used – use VACUETTE Blood Transfer Unit*
- *Slow blood flow could indicate too close contact of needle lumen and vein wall*

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Preventing Haemolysis

- **Mixing of tubes**
- *After blood collection, mix the tube content thoroughly*
- *Mix tube contents gently, do not shake*
- *Insufficient or delayed mixing of the blood with the additives in the tube can cause micro clots or interfere with coagulation*

- **Preparation of samples - Centrifugation**
- *Ensure the serum is devoid of clotting factors before centrifugation*
- *Avoid delayed separation of cells from serum or plasma > 3 hours*
- *Avoid too long or too high centrifugation*
- *Ensure samples are balanced out in centrifuge*
- *Ensure that the temperature is correctly set*

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Preventing Haemolysis

- **Storage until transportation**
- *Apply correct storage temperature – too high or too low temperature can destroy cell membrane*
- *Influence of temperature, heat or cold, e.g. during transport or if samples touch cooling elements*
- *Avoid cooling or freezing of whole blood*

- **Sample transportation**
- *Use appropriate transport containers. Cooling devices and temperature monitoring system available*
- *Pneumatic tube systems may create up to 10 g shear force*
- *Avoid storage of whole blood over several days at ambient temperatures*

References

- *The Haemolytic, Icteric and Lipemic Sample recommendations Regarding their Recognition and Prevention of Clinically Relevant Interferences; German Society for Clinical Chemistry*
- *Haemolysis as influence & interference factors; Prof.Dr. L.Thomas*
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- *Hemolysed Blood Samples: Best Practice for Prevention; Flinder University*
- *The effect of Pneumatic Tube transport on Clinical Material Studies Under Different Operating Conditions; A. Delbrück, H. Poschmann*
- *Emergency Transport of AS-1 Red Cell Units by pneumatic Tube System; G.Hardin, Gary Quick, Daniel J. Ladd*
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