

# Examination of hemoglobin and its metabolism

## Conjugated and unconjugated bilirubin levels

Bilirubin is a metabolite of heme due to its degradation in cells of the monocyte-macrophage system (MFS). Unconjugated (indirect) bilirubin is released into plasma and transported in complex with albumin to the liver, where conjugated (direct) bilirubin is formed in the presence of the enzyme bilirubin-UDP-glucuronyltransferase. The level of direct and indirect bilirubin reflects the degradation of heme and increases during hemolysis, the level of direct bilirubin also changes in liver diseases. Elevated levels of unconjugated bilirubin due to decreased bilirubin-UDP-glucuronyltransferase activity are observed in *Gilbert's syndrome*.

## Plasma hemoglobin level

The principle of the examination is the ability of hemoglobin to catalyze the oxidation of benzidine in the presence of hydrogen peroxide, which leads to a change in the color of the solution to green and later to blue. The color intensity is determined spectrophotometrically. Normal hemoglobin concentration is less than 5 mg / l. Non-coagulated blood collected in heparin is used for the examination. It is important to prevent hemolysis during sample collection and processing. The sample is centrifuged at 2,500 rpm. Plasma hemoglobin increases with intravascular hemolysis, but does not increase with extravascular hemolysis.

## Serum haptoglobin level

Haptoglobin is a plasma globulin that binds hemoglobin. Several methods are used to determine its concentration, e.g. electrophoresis, radial immunodiffusion, chromatography. Serum obtained from native blood is used as a sample. Decreased haptoglobin levels are present in hemolysis and liver disease, increased in inflammatory diseases and malignancies. When the binding capacity of haptoglobin is depleted during enhanced hemolysis, hemoglobin is excreted in the urine or intravascularly breaks down into heme, which subsequently oxidizes, and globin. Oxidized heme binds to hemopexin or albumin.

## Serum hemopexin level

Hemopexin is a plasma globulin whose function is to bind oxidized heme released from hemoglobin when haptoglobin's binding capacity is depleted. It is considered a more accurate indicator of intravascular hemolysis than haptoglobin levels. Electrophoresis or radial immunodiffusion is used for testing, and serum obtained from native blood is used as a sample. Decreased to zero levels are present during hemolysis. Decreased levels are also present in liver and kidney diseases, malignancies, infections, diabetes mellitus. Elevated levels are present in acute conditions (acute phase reactant).

## Serum methemalbumin level

Methemalbumin is a complex of oxidized heme and albumin, which is formed when the binding capacity of haptoglobin is depleted. Unlike hemopexin, it is not taken up by MFS cells and circulates in the serum until the heme is transferred to the hemopexin. Spectrophotometric methods are used to determine it, and native blood serum is used as a sample. Methemalbumin, like hemopexin, is considered a sensitive indicator of intravascular hemolysis.

## Determination of hemoglobin and hemosiderin in urine

When the binding capacity of haptoglobin is exceeded, part of the hemoglobin enters the urine. In the case of chronic hemolysis, hemoglobin is taken up by the renal tubule cells and, when damaged, is released in the form of hemosiderin. Hemosiderin is a storage form of iron that is formed by the aggregation of ferritin molecules with an excess of iron.

## Hemoglobin electrophoresis

Changes in the structure of the hemoglobin molecule lead to a change in its mobility in the electrostatic field, which is used to distinguish abnormal hemoglobin. Verona buffer electrophoresis (pH 8.6) and alkaline acetate electrophoresis in alkaline medium (pH 8.9) are used as a basis. If necessary, they are supplemented by additional tests (DNA analysis methods)

## Links

### Related articles

- Hemoglobin

- Haptoglobin
- Bilirubin

## Related literature

- DOBROTOVÁ, Miroslava. *Hematológia a transfuziológia : učebnica*. 1. edition. Praha : Grada ; Bratislava : Grada Slovakia, 2006. ISBN 80-8090-000-0.

## Source

- ws: Vyšetrenie hemoglobínu a jeho metabolizmu