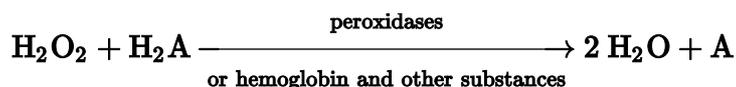


Determination of hemoglobin in urine

Hemoglobin catalyzes, like peroxidase, the oxidation (dehydrogenation) of some substrates (eg benzidine derivatives) with hydrogen peroxide:



However, it is not an enzyme activity (catalysis is conditioned by heme iron) and therefore it is not lost even after heat denaturation. We are talking about **pseudoperoxidase activity**, which is used for sensitive but non-specific evidence of hemoglobin or trace amounts of blood. It is preferable to use a chromogenic substrate to monitor the reaction, i.e., a substance that provides a markedly colored product by dehydrogenation (often benzidine or its non-carcinogenic derivatives, aminophenazone, etc.).

The reagent zone of the diagnostic strips contains a chromogen (eg tetramethylbenzidine) with stabilized hydrogen peroxide (eg cumene hydroperoxide). In the presence of free hemoglobin (**hemoglobinuria**), the indication zone turns uniformly blue. If erythrocytes are present in the urine (**erythrocyturia**), intensely green-blue dots to spots form.



Hemoglobinuria can be found in intravascular hemolysis. More damage to the glomerular membrane (**glomerular hematuria**) and bleeding from any part of the urinary tract lead to more frequent erythrocyturia. It is often found in urinary tract infections, urolithiasis and urogenital tract tumors.

In addition to hemoglobin, myoglobin also provides a pseudoperoxidase response, which can be excreted in the urine during skeletal muscle breakdown (rhabdomyolysis, crush-syndrome). The positivity of the test may also be due to peroxidases of leukocytes or certain bacteria, yeasts or fungi, which may occur in the urine, especially in urinary tract infections. To rule out the possibility of a false positive reaction due to cellular peroxidases, the reaction must be performed with boiled urine.

Contamination of the sampling vessel with strong oxidizing agents also causes a false positive reaction. On the other hand, the presence of strong reducing substances (eg ascorbic acid) can slow down or even stop the pseudoperoxidase reaction and thus cause false negative results.