Determination of catalase activity in saliva

Equipment: pipettes for 1 ml; conical measuring tube; beaker with a volume of 100 ml; tripod with test tubes. **Reagents**: 0.9% solution of NaCl; 10% p-p H2SO4; 1.5% p-p H2O2; 0.1N p-pKMnO4;

The principle of the method. The method is based on titrometric determination of the amount of hydrogen peroxide remaining in the sample after the action of the enzyme. Hydrogen peroxide is titrated with 0.1 N KMnO4 solution.

Work progress: In two test tubes (control and experimental), 1 ml of 0.9% NaCl solution, 0.3 ml of 1.5% H2O2 solution and 0.5 ml of undiluted saliva are added. The experimental sample is left for 15 minutes at room temperature, and immediately after the addition of saliva, 3 ml of 10% H2SO4 are poured into the control sample and titrated with 0.1 N KMnO4 solution until a slightly pink color appears, which does not disappear for 30 seconds. After 15 minutes of incubation, 3 ml of 10% H2SO4 solution is also added to the experimental sample and titrated in the same way as the control one.

The calculation of the activity of catalase is carried out according to the formula:

 $X = (K - O) \times 0.3 \text{ mg H2O2} / \text{ml per minute}$, where K is the amount of KMnO4 used for titration of the control, O is the amount of KMnO4 used for titration of the experiment, 0.3 - coefficient taking into account the H2O2 titer, the amount of saliva in the sample and the incubation time.

Clinical and diagnostic significance of determining the activity of catalase: Catalase is found in all tissues and body fluids, but it is especially abundant in the stroma of erythrocytes and liver. In the process of oxidation of some substances, hydrogen peroxide is formed, which is toxic to the body. Catalase breaks down hydrogen peroxide into molecular oxygen and water. Determination of catalase activity is important for the diagnosis of cancer, anemia, and tuberculosis. In these diseases, the activity of catalase decreases.

In the laboratory, the test samples are titrated with a 0.1e solution of KMnO4 solution until the color indicated below appears, which does not disappear for 30 seconds or more.



Write down in a notebook the volume of KMnO4 used for titration of the control and experimental samples (in ml)

Variants	Volume of KMnO4 (in ml) used for titration control sample (K)	Volume of KMnO4 (in ml) used for titration of the experimental sample (O)
Variant 1	35	15
Variant 2	15	10
Variant 3	25	15
Variant 4	15	14
Variant 5	20	11
Variant 6	35	2
Variant 7	23	10