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OPTIMIZATION AND VERIFICATION OF THE METHOD FOR DETERMINING THE ANTIOXIDANT ENZYME CATALASE IN VITRO

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The oxidative stress is unavoidable consequence on aerobic life. The organism constantly controls the presence on pro-oxidants and antioxidants. The balance between them is closely regulated and that is very important for keeping the vital cell functions

The cell is exposed on attacks from different external and internal forms on reactive oxygen types. The hydrogen peroxide is compound in the biosphere which is one of the most common reactive oxygen types

The hydrogen peroxide is present in the environment as nus-product from anaerobic metabolism, respiratory and photosynthetic electron transport chains or such as product from enzymatic activity on oxidases

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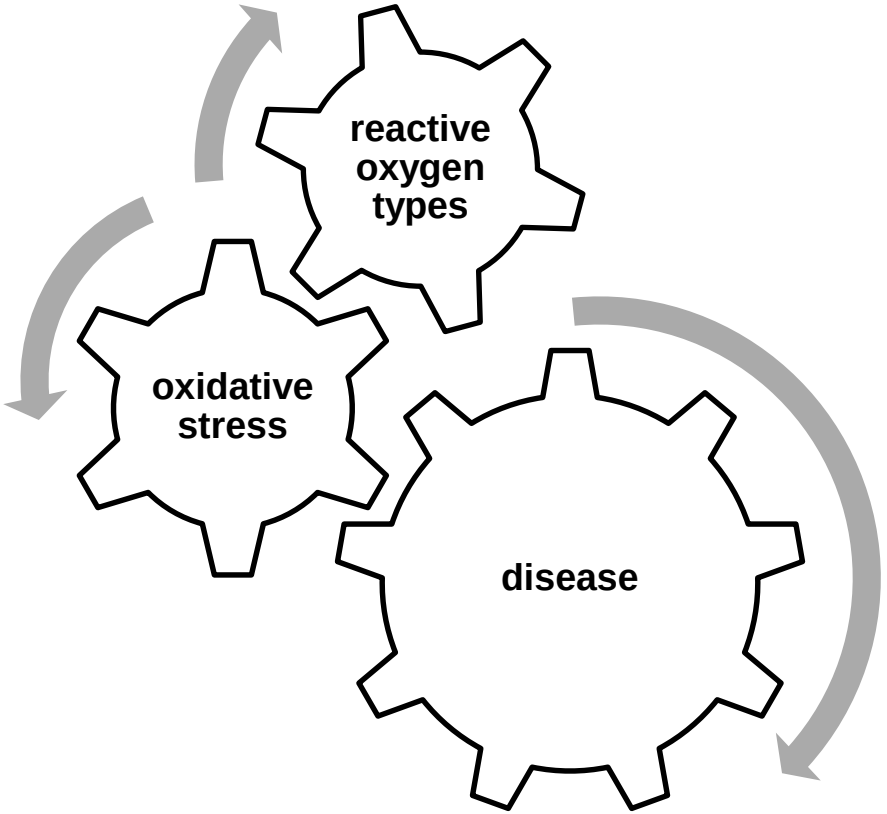
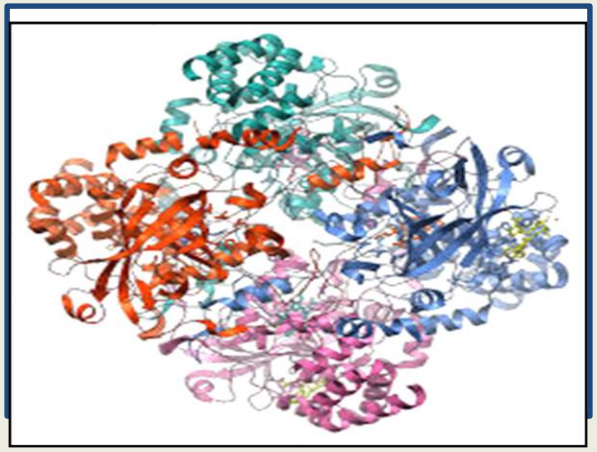


On the other side, an antioxidant enzyme catalase is well studied because of its important function in cell protection from the potentially toxic effects of hydrogen peroxide.



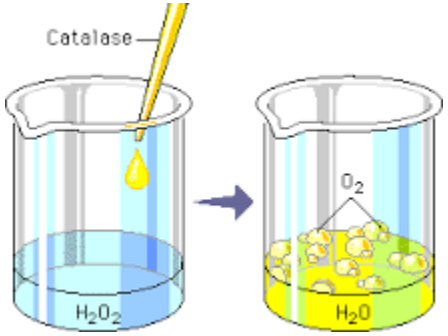
Three-dimensional structure of the enzyme catalase

Dysfunctional catalase in the body may cause a disease that is called *acatalasemia or takahara*.

There are a few published methods for measuring the catalase activity in different tissues and organs. The determination on catalase activity can be made by

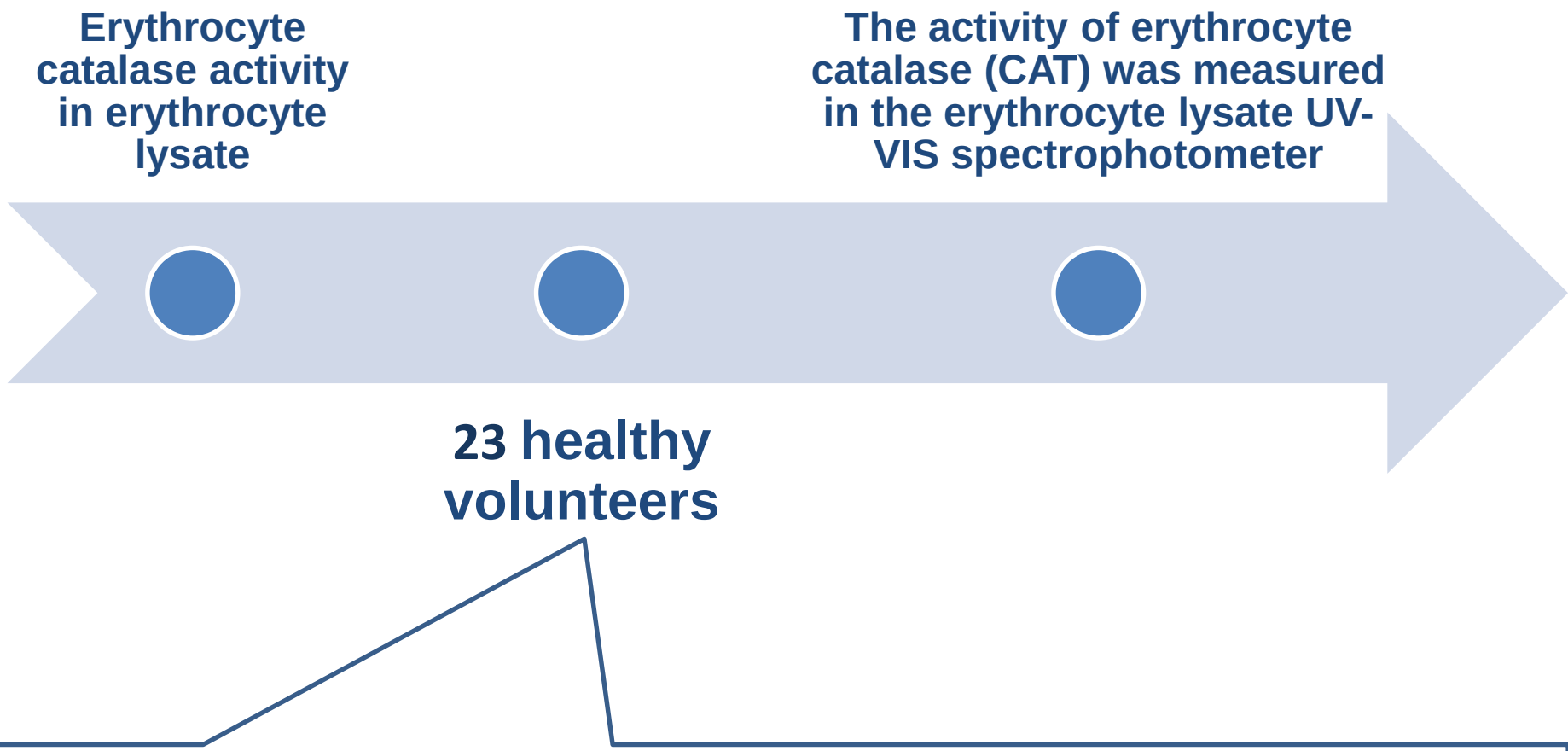
- manometric
- Gasometric
- ungasometric method.

Determine the activity on erithroid catalase in hemolysate on 25 ºC with Aebi, 1984 method.



The speed on decomposition on the supstrat - hydrogen peroxide (H_2O_2), spectrophotometric method, on wavelength on 240nm during 30s

Materials and method



- ✓ Department of Occupational Medicine in the Institute of Public Health of the Republic of Macedonia
- ✓ Specific exclusion criteria considered for the study were the presence of liver dysfunction, diabetes mellitus, heart failure or renal failure; oral antioxidant supplementation at the moment of the enrollment

The method was optimized in order to achieve

- the optimal sample dilution

- Optimal time point for measuring the decrease in the absorbance due to the substrate decomposition

Table 1. Preparation on working standard solutions on catalase

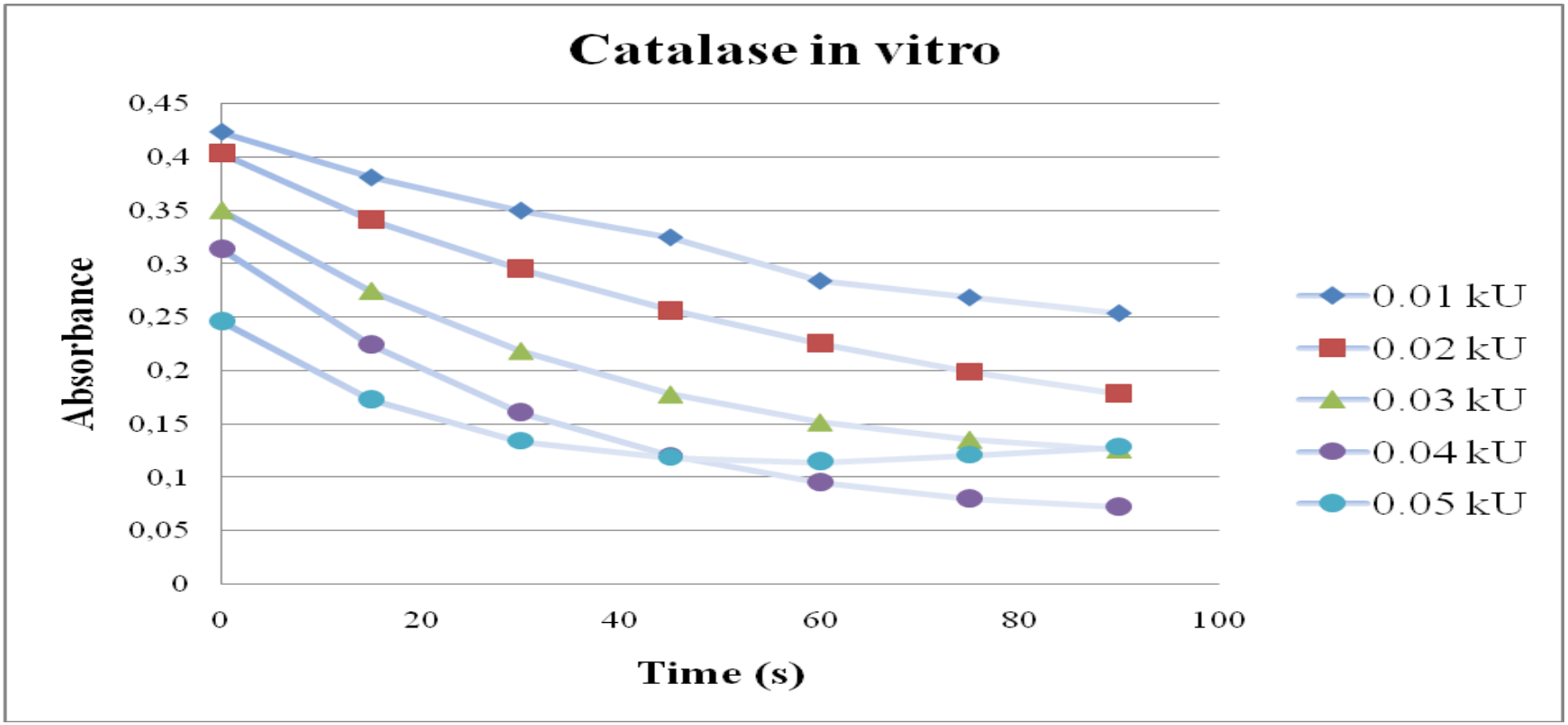
Concertation of standards	Volume of stock solution 2 (0,5 KU/ ml)	Volume of water
0,01 KU/ ml	0,1 ml	ad 5 ml
0,02 KU/ ml	0,2 ml	ad 5 ml
0,03 KU/ ml	0,3 ml	ad 5 ml
0,04 KU/ ml	0,4 ml	ad 5 ml
0,05 KU/ ml	0,5 ml	ad 5 ml

After we prepared the working standard solutions on catalase we have measured activity on working standard solutions on catalase after 0, 15, 30,45,60,75 and 90 s.

Table 2. Measured activity on working standard solutions on catalase after 0,15s, 30s, 45s, 60s, 75s и 90s.

Time (s)	0,01 KU	0,02 KU	0,03 KU	0,04 KU	0,05 KU
0	0,4237	0,4039	0,3501	0,3147	0,2471
15	0,3812	0,3415	0,2747	0,2247	0,1732
30	0,3502	0,2956	0,2185	0,1613	0,1343
45	0,3252	0,2565	0,178	0,1203	0,1186
60	0,2845	0,2256	0,1519	0,0957	0,1147
75	0,269	0,199	0,1356	0,0802	0,121
90	0,2544	0,1785	0,1264	0,0724	0,1287

Figure 1. Measured activity on working standard solutions on catalase after 0,15s, 30s, 45s, 60s, 75s и 90s.



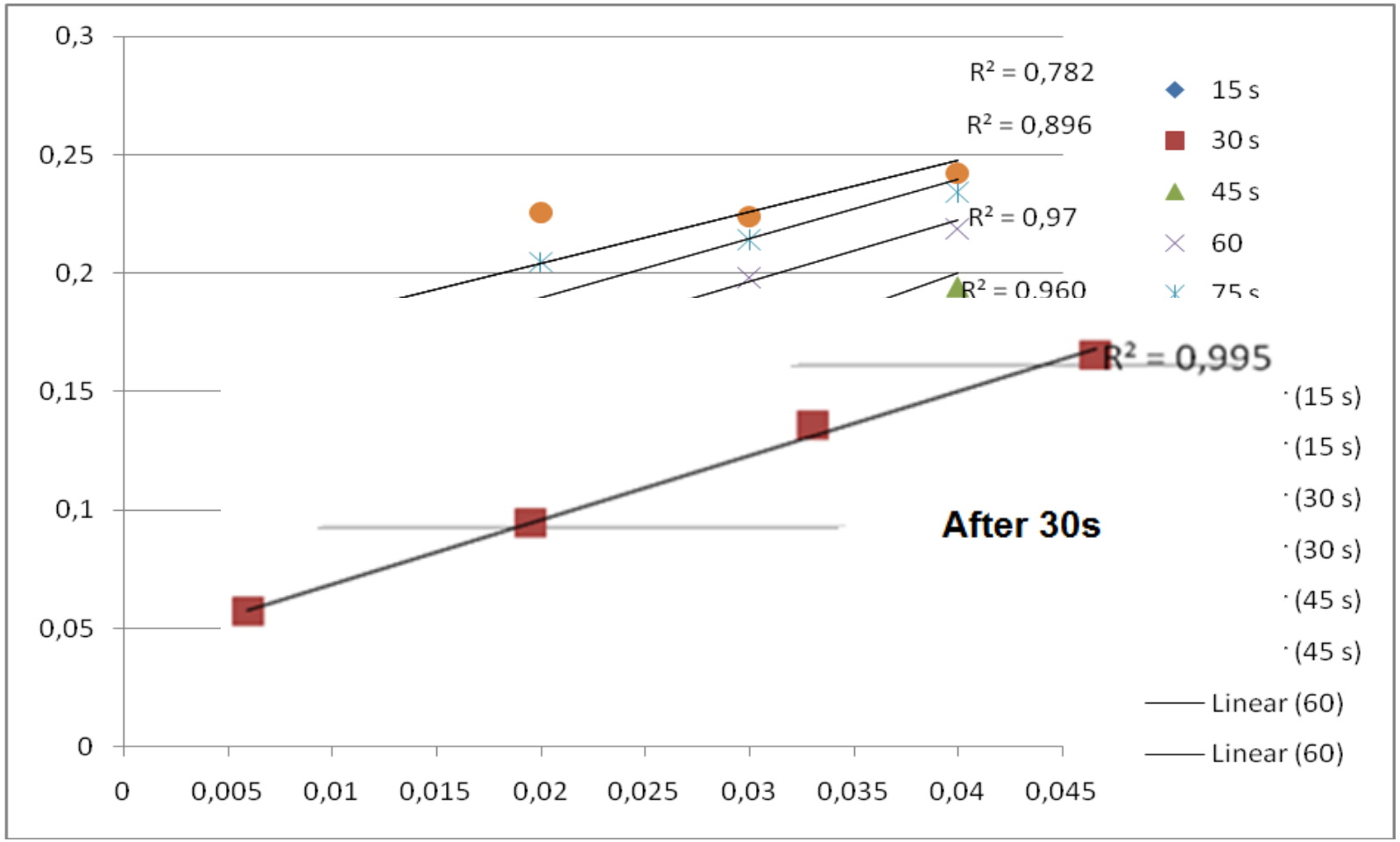
The decomposition of hydrogen peroxide as a substrate is proportional to the standard solutions of catalase concentration to 0,04 kU, while linearity was not demonstrated for a standard solution of 0,05 kU / ml.

In order to select the optimum time for reading the reaction, the change in absorbance of the standard solutions was determined at the beginning of the reaction, after 15 s, 30 s, 45 s, 60 s, 75 s and 90 s.

Table 3. Change in absorption on standard solutions

	Change in absorption					
Conc. (KU/ ml)	0 - 15 s	0 - 30 s	0 - 45 s	0 - 60 s	0 - 75 s	0 - 90 s
0,01	0,0425	0,0735	0,0985	0,1392	0,1547	0,1693
0,02	0,0624	0,101	0,1474	0,1783	0,2049	0,2254
0,03	0,0754	0,1316	0,1721	0,1982	0,2145	0,2237
0,04	0,09	0,1534	0,1944	0,219	0,2345	0,2423

Figure .2 Change in absorption on standard solutions

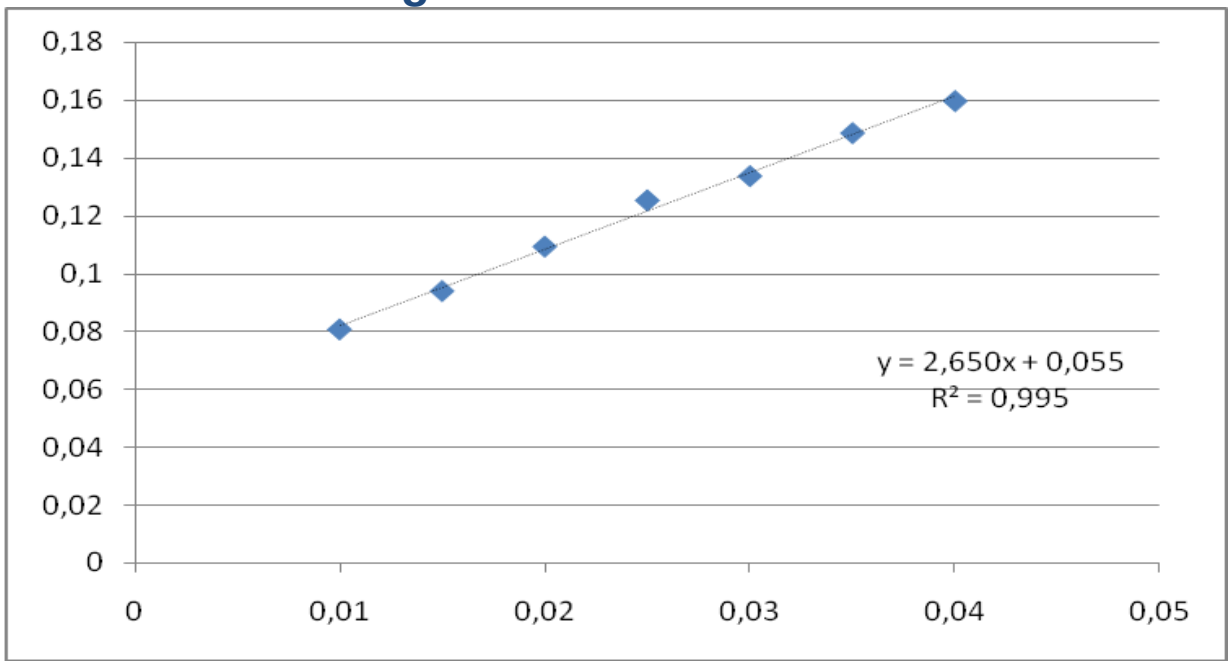


As we obtained the best correlation factor when measuring the decomposition of hydrogen peroxide 30 s after the initiation of the reaction (R2 = 0.995), we chose to measure the change in absorbance in two time points, immediately and 30 s after addition of the substrate in the reaction mixture

Table 4. Constructing a standard curve for catalase

Standard solution	Cocentration (KU/ml)	Change in Abs (30 s)
1	0,01	0,0807
2	0,015	0,094
3	0,02	0,1094
4	0,025	0,1254
5	0,03	0,1338
6	0,035	0,1487
7	0,04	0,1598

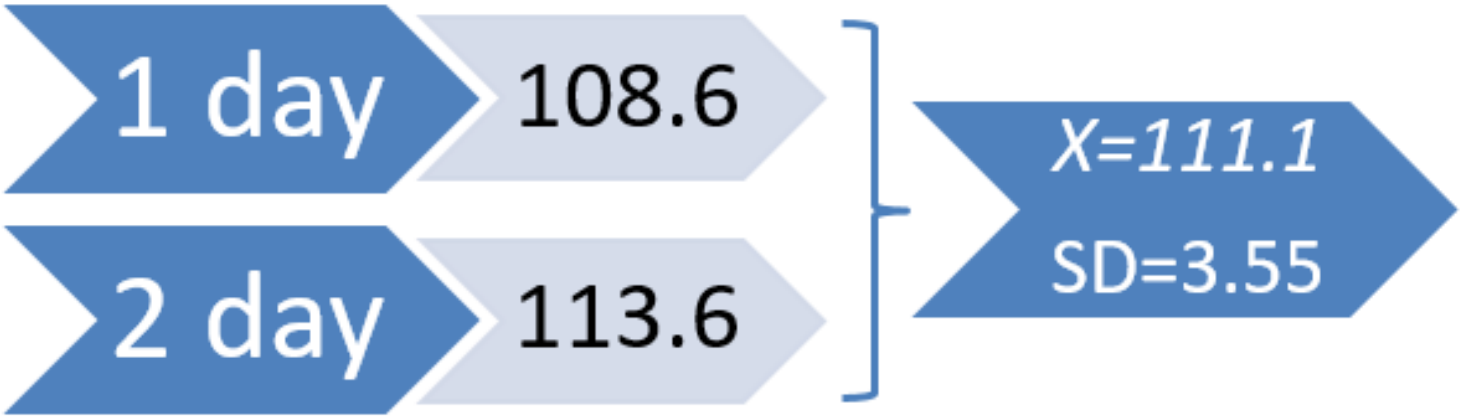
Figure 3. Standard curve for catalase



The choice of standard concentrations of catalase to construct a calibration curve was made on the basis of literary knowledge of the expected concentration / activity of catalase in the serum of healthy subjects

Number of patients	Change in Abs (30 s)	catalase (KU/ ml)
1	**0.1034**	**90.7**
2	**0.1481**	174.6
3	**0.1261**	133.4
4	**0.1038**	91.4
5	**0.1133**	109.3
6	**0.1176**	117.4
7	**0.1135**	109.6
8	**0.1001**	84.4
9	**0.1244**	130.1
10	**0.1338**	147.7
11	**0.1511**	180.3
12	**0.1076**	98.6
13	**0.1174**	117
14	**0.1076**	98.6
15	**0.1181**	118.3
16	**0.0907**	66.7
17	**0.1044**	92.5
18	**0.0969**	78.5
19	**0.1073**	98
20	**0.088**	61.8
21	**0.1076**	98.6
22	**0.0997**	83.7
23	**0.1174**	117
Mean value (KU/ ml)		108,6

Number of patients	Change in Abs (30 s)	Catalase (KU/ml)
1	0,1091	**101.3**
2	0,1283	**137.4**
3	0,1382	**156**
4	0,1106	**104.3**
5	0,1181	**118.3**
6	0,1207	**123.2**
7	0,1186	**119.2**
8	0,1079	**99.1**
9	0,1293	**139.3**
10	0,1230	**127.5**
11	0,1386	**176.7**
12	0,1129	**108.5**
13	0,1177	**117.5**
14	0,1250	**131.2**
15	0,1171	**116.4**
16	0,1089	**101**
17a	0,1118	**106.5**
18	0,0998	**83.9**
19	0,1008	**85.9**
20	0,0742	**55.8**
21	0,1266	**104.3**
22	0,0989	**82.2**
23	0,1338	**117.7**
Mean value (KU/ ml)		113.62



The t-test showed that the method is reproducible (there is no statistically significant difference in the results obtained in two consecutive days of measurement, p = 0,1643).

Conclusion

The performance verification of the method was assessed through the determination of two parameters:

- **Linearity**
- **Reproducibility**

The results proved that the method is reproducible and linear in the concentration range of 0.01 KU/ml – 0.04 KU/ml.

Thank You for Your Attention!