TOS | Total Oxidant Status

Summary and Explanation

Reactive oxygen and nitrogen species are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms that remove them via enzymatic and non-enzymatic antioxidative mechanisms. Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative or in over 100 disorders, develops1 . Serum (or plasma) concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive and costly and require complicated techniques2 . Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant status (TOS) of a sample is measured and this is named total peroxide (TP)1,3,4,5 , serum oxidation activity (SOA)6 , reactive oxygen metabolites (ROM)7 or some other synonyms.

Principle of Assay

Oxidants present in the sample oxidize the ferrous ion– chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Equiv./L)8 .

Reagent Composition

Content		Concentration
Reagent 1	Buffer Solution	
	H_2SO_4	25mM pH1.75
Reagent 2	Substrate Solution	
	H_2SO_4	25mM pH1.75
	Ferrous ion	5 mM
	O-dianisidine	10nM
Standard	H_20_2	10 µmol/L
QC Level 1	H_20_2	5 µmol/L
QC Level 2	H_20_2	20 µmol/L

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C is recommended. Stable up to expiry date when stored capped and at 2–8 °C even after start using.

Normal Range

Human Serum: 4.00 – 6.00 µmol/L (400 – 600 umol/hL)

Each laboratory is recommended to establish their own reference values.

Performance Characteristics

Precision

Inter-assay coefficent of variation 3.2%

Intra-assay coefficent of variation 3.9%

Assay Range

Samples containing 0.2 – 80 µmol H_20_2 Equiv. /L can be assayed without further dilution or concentration.

Interferences

EDTA interfere with the results.

Sample

Blood serum, heparinised plasma, semen plasma, saliva, urine, cell lysates and tissue homogenates can be used as sample.

Serum samples are stable up to 1 week stored at 4˚C, 6 months at -20˚C and 1 year at -80˚C.

Safety Precautions and Warnings

1. For in vitro diagnostic use only.

2. Do not pipette by mouth.

3. Exercise the normal precautions required for handling laboratory reagents.

4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.

5. Do not use reagents beyond the expiry date.

6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

7. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.

8. Health and safety data sheets are available on request.

Procedure

Wavelength	530nm
Pipette into cuvette as below order	
Sample OR Standard OR H_20	45 µl
Reagent 1	300 µl
Mix well	
Read absorbance (A1) after 30 seconds	
Reagent 2	15 µl
Mix well	
Read absorbance (A2) after 5 minutes at 37˚C OR	
Read absornabce (A2) after 10 minutes at RT	

Calculation

A2 – A1 = ΔAbs of standard or sample

$$\text{Results} = \frac{\Delta\text{Abs Sample}}{\Delta\text{Abs Standard}]} \times 10^{*}$$

*Concentration of standard

References

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Third ed. Oxford: Oxford Science Publications; 2000. p. 617–24.

2.Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol, Regul Integr Comp Physiol 2004;286(3):R431–44.

3.Yeni E, Gulum M, Selek S, et al. Comparison of oxidative/antioxidative status of penil corpus cavernosum blood and peripheral venous blood. Int J Impot Res 2005;17(1):19–22.

4.Yanik M, Erel O, Kati M. The relationship between potency of oxidative stress and severity of depression. Acta Neuropsychiatr 2004;16(4):200–3.

5.Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatiform mole. Swiss Med Wkly 2003;133(41–42):563–6.

6. Nakamura K, Endo H, Kashiwazaki S. Serum oxidation activities and rheumatoid arthritis. Int J Tissue React 1897;9(4):307–16.

7. Ceylan E, Gulsun A, Gencer M, Aksoy N. A new parameter in the detection of tuberculosis activity: reactive oxygen metabolites. Respiration 2005;72(2):156–9.

8. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005 Dec;38(12):1103-11.

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