# **YÖNTEMLER**

## **TOTAL ANTIOXDANT STATUS (TAS)**

TAS levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2 ' - Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L (Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radicalcation. Clin Biochem 2004;37:277-85.)

## **HDL**

Enzymatic colorimetric test

- Sample and addition of R1
- Addition of R2 and start of reaction

In the first step LDL, VLDL and Chylomicrons are eliminated and transformed to non reactive compounds and specific condition for the reaction. By the second reagent only the HDL-Cholesterol is subject to color reaction

Cholesterol Esterase

# <u>Glukoz</u>

Enzymatic colorimetric test on basis of Trinder – Reaction:

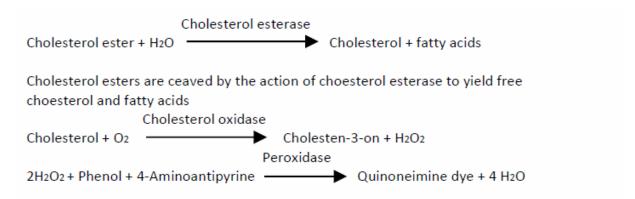
Glucose oxidase

Glucose + 
$$O_2$$
  $\longrightarrow$  Gluconic acid +  $H_2O_2$ 

Peroxidase

 $2H_2O_2$  + Phenol + 4–Aminoantipyrine  $\longrightarrow$  Red Quinoneimine +  $4H_2O_2$ 

## **Total Kolesterol**



Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A<sup>4</sup>-Cholestenone and hydrogen peroxide. Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

## **Total Protein**

# Test principle:

Colorimetric assay, Sample and addition of Reagent start of the reaction: Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents auto reduction of copper.

The color intensity is directly proportional to the protein concentration which can be determined photometrically.

#### **Trigliserid**

# Triglycerides in the sample originates, by means of the coupled reactions described below, acoloured complex that can be measured by spectrophotometry. Triglycerides +H<sub>2</sub>O | lipase | Glycerol + Fatty acids Glycerol + ATP | glycerolkinase | Glycerol - 3 - P + ADP | Glycerol - 3 - P + O<sub>2</sub> | G-3 - P - oxidase | Dihidroxyacetone - P + H<sub>2</sub>O<sub>2</sub> | 2H<sub>2</sub>O<sub>2</sub> + 4 - Aminoantipyrine + 4 - Chlorophenol | G-3 - P - oxidas | Quinoneimine + 4 H<sub>2</sub>O

#### <u>Lipaz</u>

# Test principle:

Enzymatic colorimetric assay;

- Sample and addition of R1 (buffer/colipase/cholate)
- Addition of R2 (emulsion/chromogenic substrate/cholate) and start of reaction:

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lipase

1,2-O-dilauryl-rac-glycero-3-

glutaric acid-(6-methylresorufin)ester

spontaneous

glutaric acid-

glutaric acid-

glutaric acid + methylresorufin

(6-methylresorufin) ester decomposition
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The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The colour intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.