**AST**

**Summary:**

Aspartate aminotransferase (glutamate-oxaloacetate-transaminase) belongs to the transaminases, which catalyze the interconversion of amino acids and a-keto acids by transfer of amino groups. Aspartate aminotransferase is commonly found in human tissue. Although heart muscle is found to have the most activity of the enzyme, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys. AST is present in both the cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of AST is that from the cytoplasm, with a smaller amount coming from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, and organ damage. The International Federation of Clinical Chemistry (IFCC) recommended in 1977 and 1980 standardized procedures for AST determination, including optimization of substrate concentrations, employment of TRIS\* buffers, preincubation of combined buffer and serum to allow side reactions with NADH to occur, substrate start, and optional pyridoxal phosphate activation. In 2002 the IFCC confirmed their recommendation and extend it to 37°C. This method is derived from the IFCC reference method. \*TRIS = Tris(hydroxymethyl)- aminomethane

**ALT**

**Summary:**

Alanine aminotransferase (glutamate-pyruvate-transaminase) belongs to the group of transaminases which catalyze the conversion of amino acids to the corresponding a-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy, and damage to internal organs. Increased ALT activity in the serum, however, is a rather specific indicator of damage to the liver parenchyma, while AST is not necessarily a liver-specific parameter. The International Federation of Clinical Chemistry (IFCC) recommended standardized methods for the determination of ALT with optimized substrate concentrations, use of TRIS buffer, simultaneous preincubation of serum with buffer (to avoid competing reactions with NADH), substrate start, and pyridoxal phos-phate activation.

The method described here is derived from the IFCC reference method.

**Test principle:**

UV test according to the IFCC method.

ALT

L-Alanin + 2-Oxoglutarate ⎯⎯ L-Glutamate + Pyruvat

LDH

Pyruvat + NADH + H+ ⎯⎯ D-Lactate+ NAD+

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase, ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the tran- saminase reaction between L-Alanine and 2-Oxoglutarate. The pyruvate formed, is reduced to lactate in the presence of LDH. As the reactions proceed, NADH is oxidized to NAD+. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm.

**HDL**

**Summary:**

HDL (High Density Lipoproteins) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are secreted into the intestine via the biliary tract. Monitoring of HDL- cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL- cholesterol concentrations and the risk of atherisclerotic disease. Elevated HDL- cholesterol concentrations are protective against coronary heart disease, while reduced HDL- cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk. A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, and precipitation-bases methods. Of these precipitation-based methods are used routinely. HDL cholesterol is first separated by precipitating apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride. Such precipitation -bases method are, however, time consuming and not amenable to automated analysis. Thus, there is a great clinical need for a convenient and reliable method for measuring HDL-cholesterol in serum without any pretreatment. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanionmetal combinations and the use of polyethylene glycol (PEG) with antiapoprotein B and anti-apoprotein CIII antibodies. **Test principle:**

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

Cholesterol ester + H2O Cholesterol + fatty acid

Cholesterol Oxidase

Cholesterol + O2 Cholesten-3-on + H2O2

Peroxidase

H2O2 + phenol + 4-aminoantipyrine quinoneimine dye+4 H2O

**LDL**

Düşük yoğunluklu lipoproteinler (LDL), arteroskleroz ve koroner skleroz ilerlemesinde önemli rol oynarlar. LDL, trigiliseridce zengin olan VLDL’ den ( Çok Düşük Yoğun Lipoprotein) lipolitik enzim çeşitleri tarafından karaciğerde sentezlenir. Plazmadaki LDL, karaciğerde parankima hücrelerindeki özel almaçlar tarafından seçilir. Kandaki yükselmiş olan LDL yoğunluğu artan biyolojik modifikasyonlarla birleştiğinde endotelyal fonksiyonlara zarar verir ve daha yüksek LDL yoğunluğu damar duvarlarındaki monosit/makrofaj sistemindeki düz kas hücrelerini kaplar. Arterosklerotik plaklar da gizlenmiş olan kolesterolün ana kaynağı LDL’ dir. Koroner arteroskleroz tanısında LDL kolesterol tek başına güçlü bir klinik belirleyicidir. Bu nedenle, öncelikli olarak lipid azaltıcı tedavilere odaklanmalı daha sonra arterosklerozisde değişen endotelyal fonksiyonlarını korumalı bunun yanı sıra plakların kırılmasını önleyici süreç izlenmelidir. LDL kolesterol tanımlamada çeşitli metodlar yaygındır; örneğin ultrasantrifüj, referans metod, lipoprotein elektroforezi ve filtrasyon metodu. Filtrasyon yöntemi apolipoprotein-B bulundurur, LDL kolesterol filtrasyonunu hızlandırmak için polivinil sülfat, dekstran sülfat ya da polisiklik anyonlar kullanılır. LDL-kolesterol içeriği genellikle polivinil sülfat ve dekstran sülfat ile filtrasyon sonrası süpernatant içinde kalan toplam kolesterol ve kolesterol arasındaki fark (VLDL-ve HDL-kolesterol) hesaplanır. Klinik lipid araştırmacıları iki değerlikli katyonların varlığında polianyonlar kullanılan ultrasantrifügasyon ve filtrasyon yöntemleri önerir. Filtrasyon yöntemleri ancak zaman alıcı olduğundan, otomasyona uygulanamaz ve lipemik serum, yüksek serbest yağ asidinden etkilenebilir. Birçok LDL kolesterol tanımlama yönteminde örnek immunoadsorbsiyon ve santrifüj tabii tutulur. Friedewald formülü LDL-kolesterol yoğunluğu hesaplanmasın da yaygın olarak uygulanmaktadır. Formülle 2 kolesterol tanımlanır, 1 trigliserid belirlenmesi hem de HDL partiküllerinin filtrasyonun da ve açlık kan örneklerin de VLDL-kolesterol ve trigliserid arasında doğrudan ilişkili olduğu tahmin edilebilir. Az miktarda şilomikron ve normal olmayan lipoprotein varlığında formul LDL kolesterol değerini yanlış verir. Bundan dolayı ön hazırlık adımları ya da hesaplama yapmadan, basit ve kararlı LDL kolesterol ölçümüne ihtiyaç vardır.

**Cholesterol**

Summary:

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is syn- thesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for athero- sclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/ concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of A4 cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods. The Analyticon cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3% for both precision and bias.

Test principle:

Cholesterol esterase Cholesterol ester + H2O Cholesterol + fatty acids

Cholesterol esters are ceaved by the action of choesterol esterase to yield free

choesterol and fatty acids Cholesterol oxidase Cholesterol + O2 Cholesten-3-on + H2O2

Peroxidase

2H2O2 + Phenol + 4-Aminoantipyrine Quinoneimine dye + 4 H2O

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A4- 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.

**Uric acid**

Summary:

Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renai and metabolic disorders, including renal failure, gout, leukaemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs. The oxidation of uric acid provides the basic for two approaches to the quantitative determination of this purine metabolite. One approach is the reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.

A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidise uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric method.

The assay described here is a slight modification of the colorimetric method. The modifications were described by Siedel. The peroxide reacts with phenole and amino-antipyrine in the presence of peroxidase to form a quinonimine dye. The intensity of the red color is proportional to the uric acid concentration and is determined photometrically.

Test principle:

Colorimetric endpoint assay

Uricase

Uric acid + 2 H2O + O2 Allantoin + CO 2 + H 2O 2

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

POD

2H2O2 + 4H+ + phenole + 4-aminoantipyrine quinonimine dye + 4H2O

The increase in absorbance is measured.

**Glucose**

Summary:

Glucose is the central energy source of the cells in the organism. The most common supply follows hydrolytic cleavage of polymeric carbohydrates, in general starch. Glucose is a monosaccharide with an postprandial concentration of 5 mmol/l in the blood and serves as an indispensable energy-supply for cellular functions. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation.

Glucose regulations become executed the diagnosis and course control of carbohydrate metabolism illnesses like the diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and with insulinoma.

The test bases on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in hydrogen peroxide, which is subsequently used for the generation of a coloured product by peroxidase. In the Trinder method the carcinogenic ortho-dianisidine used in earlier formulations has been replaced by phenole and 4-amino-antipyrine.

Test principle:

Enzymatic colorimetric test on basis of Trinder – Reaction:

Glucose oxidase Glucose + O2 Gluconic acid + H2O2

Peroxidase

2H2O2 + Phenol + 4–Aminoantipyrine Red Quinoneimine + 4H2O

**Triglycerides**

**Test principle:**

Triglycerides in the sample originates, by means of the coupled reactions described below, acoloured complex that can be measured by spectrophotometry.

Triglycerides + H2O lipase Glycerol + Fatty acids

Glycerol + ATP glycerol kinase Glycerol – 3 – P + ADP

Glycerol – 3 –P + O2 G-3-P-oxidase Dihidroxyacetone – P +H2O2

2 H2O2 + 4 – Aminoantipyrine + 4 – Chlorophenol G-3-P-oxidas Quinoneimine + 4 H2O