Part two:

Introduction to Clinical Chemistry Key

Concepts

1. Clinical chemistry tests measure concentrations or activities of substances (ions, biomolecules, and complexes) in body fluids.
2. These tests may use different kinds of body fluids such as whole blood, plasma, serum, urine and cerebrospinal fluid.
3. The medical interpretation of a test result is based on comparison to a reference interval that typically reflects the range of values expected for healthy people or a medical decision level (MDL) (Normal value) for the diagnosis and treatment of disease.

Clinical Chemistry

Clinical chemistry is the application of biochemical and physiological knowledge to determine the health status of a person by providing general information's about biochemical profiles of diseases to help in early detection, diagnosis, treatment or prevention of diseases. The great majority of clinical chemistry analysis is performed on blood (whole blood, serum or plasma).

Most methods in clinical chemistry are based on quantitative measurement of a colored compound produced when the sample contains the substance to be measured, which is mixed with an appropriate reagent to produce a characteristic color. The measurements are made with instrument called spectrophotometer (wave length of visible light). The intensity of color produced is proportional to the amount of substance being measured.

Spectrophotometer

Spectrophotometer is an instrument of absorbance measurement of a solution at one or more wave length. The spectrophotometer produces light of limited wave length for interaction with the sample.

In order to determine the concentration of a light-absorbing analyte in solution, a spectrophotometer measures light transmitted by that analyte in solution. Such an analyte may absorb, transmit, and reflect light to varying degrees, but always of a characteristic nature for the analyte.

α–Amylase

Determination of pancreatic α–Amylase Activity

Plasma contains two isoenzymes of α-amylase: Pancreatic (P-type): secreted by pancreas and Salivary (S-type) produced by salivary glands. In norm, pancreatic amylase constitutes 40% of total serum amylase activity, and salivary 60%.

Determination of α-amylase activity is very important for diagnosis of pancreatic pathology. Two times and more increased activity of α-amylase strongly indicates pancreatic damage.

AMYs in human serum have pH optimum at 6.9 to 7.0. AMYs normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass through the glomeruli of the kidneys, and AMY is the only plasma enzyme normally found in urine. AMYs are present in a number of organs and tissues. The greatest concentration is noted in the salivary glands, which secrete a potent AMY (S-type) to initiate hydrolysis of starches while the food is still in the mouth and esophagus. In the pancreas, the enzyme (P-type) is synthesized by pancreatic cells and then is secreted into the intestinal tract by way of the pancreatic duct system. In the intestinal tract, effective action of pancreatic and

intestinal AMY is favored by mildly alkaline conditions in the duodenum. Intestinal maltase then further hydrolyzes maltose to glucose. The enzyme present in normal serum and urine is predominantly of pancreatic (P-AMY) and salivary gland (S-AMY) origin.

Clinical Significance

In acute pancreatitis, α-amylase activity in the blood and urine increases 10-30 times. Initial increase of α-amylase activity is observed within 4-6 hours after the beginning of the disease, reaches peak within 12-24 h. then decreases and returns to Norm within 2-6 days.

Blood AMY activity is physiologically low and constant and greatly increases in acute pancreatitis and salivary gland inflammation. In acute pancreatitis, a rise in serum AMY activity occurs within 5 to 8 hours of symptom onset; activities typically return to baseline by the third or fourth day. A portion of the clearance of AMYs from the circulation occurs via renal excretion into the urine. Biliary tract diseases, such as cholecystitis, cause up to a fourfold increase in serum P-AMY activity.

Principle

In this direct method, α-amylase catalyzes the hydrolysis of 2-chloro-p-nitrophenylα-D-maltotrioside (CNP-G3) substrate at pH 6.0 forming 2-chloro-p-nitrophenol (CNP) and free glycosides. The reaction is monitored kinetically at 405 nm by the rate of formation of the colored CNP produced, proportional to the activity of the αamylase in the sample.

Procedure 1. Preincubate working reagent, samples and controls to reaction temperature.

2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	ºC	
R1.Monoreagent Serum/plasma Urine	1.0 mL 20 µL –	1.0 mL – 10 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Calculations

Serum, plasma

U/L = ΔA/min x 3591

Reference values

Serum, plasma

< 86 U/L (1.43 µkat/L)