DETERMINATION OF THE CATALYTIC CONCENTRATION OF LACTATE DEHYDROGENASE

PRACTICE 4:

DETERMINATION OF THE CATALYTIC CONCENTRATION OF LDH USING KINETIC METHOD FOR ELISA READER

THEORETICAL PREPARATION FOR EXERCISE

Lactate dehydrogenase (LDH) - its occurrence, biochemical function in organism, diagnostic significance. LDH isoenzymes. The principle of enzyme kinetic measurements of LDH and its significance.

PRINCIPLE OF THE METHOD

Lactate dehydrogenase (LDH) catalyzes the oxidation of lactate using NAD + to yield pyruvate and NADH. The catalytic concentration is determined from the rate of increase of NADH, which is measured spectrophotometrically.

REAGENTS - composition:

Reagent (A) : N-Metyl-D-glucamin 0,406 mol/l, lactate 62,5 mmol/l, pH 9,4
Reagent (B) : NAD^+ 50 mmol/l

MATERIAL - samples

Students will learn the principles of analysis on the ELISA reader. They will prepare solutions of reagent and sample calibrator LYO-KAL (BIO-LA-TEST).

The analysis of the catalytic concentration takes place under predetermined conditions (incubation at 37 ° C at intervals of three minutes) on the ELISA reader. The analysis will take place automatically after programming the device. The method used - kinetic measurements in time.

After the expiry of the analysis, students recall the stored analysis results and use them to create the protocol of measurement.

PREPARATION AND IMPLEMENTATION

Preparation of working Reagent (R):

Pour the contents of the vial B into the bottle A. Mix properly. Smaller volumes can be prepared by mixing 4 mL of Reagent A + 1 mL of Reagent B. Reagent is stable for 3 days at 2-8 ° C.

Prepare only the amount of working reagent, which is needed for a specific number of your samples for the whole group. It's necessary to calculate the required amount.

EVALUATION

Use the log analysis results from ELISA reader please for the protocol. Compare the results of the determination of catalytic concentration of lactate dehydrogenase in the calibrator with attested value. In conclusion evaluate the benefits of an automated analyzer for the laboratory. Explain, what U/l unit expresses.

$$\frac{\Delta A_{340}/\text{min} \times V_t \times 106}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300 and the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.025, the sample volume (Vs) is 0.025 and 1 U/L are 0.01667 μkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA340/min x 6508= U/L

ΔA340/min x 108= μkat/L

Reference values - human:

LDH : 132-228 IU/l = 2,20-3,80 μkat/l

Increase: patients with liver disease, renal disease, myocardial infarction, many malignant diseases, progressive muscular dystrophy.