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ACID PHOSPHATASE

FREEZE DRIED

Cat. No.: 42401 20x3 ml 42411 12x10 ml

Reagent kit for determination of acid phosphatase activity in serum (Hillmann's colorimetric method).

Acid phosphatase consists of several isoenzymes originating from different tissues. Most of the enzymes are found in lysosomes. Isoenzymes differ from one another in their catalytic activity, substrate specificity and sensitivity to inhibitors. All isoenzymes are labile at alkaline pH. From diagnostic point of view the **isoenzyme of prostatic origin** is of primary importance which in contrast to that of erythrocytic origin present in high concentration in serum **can be inhibited by tartrate**. Immunochemical methods (RIA, FIA, ELISA, immunoelectrophoresis) have recently been developed that allow the estimation of the concentration of isoenzymes instead of their activities. Monovalent immunosera do not cross react with different isoenzymes of acid phosphatase. Increased activities of total and specific prostatic acid phosphatase are associated with prostate disorders, prostatic cancer and its metastases. Elevations in total enzyme activity are observed also in cases of myeloma, thrombocytopenia and some liver diseases.

Principle

Acid phosphatase present in serum splits α-naphthyl phosphate into α-naphthol and inorganic phosphate. The α-naphthol deliberated during the reaction gives a diazo-dyestuff with Fast Red TR. The enzyme fraction of prostatic origin (prostatic acid phosphatase) can be inhibited by tartrate.

Reference values

Total: Male: **max. 10 U/l (0,17 µkat/l)**
Female: **max. 7.8 U/l (0,13 µkat/l)**
Prostatic: **max. 4.3 U/l (0,072 µkat/l)**
It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent (R1)
Citrate buffer, pH=5.20 150 mmol/l
2. Reagent (R2)
α-Naphthyl phosphate 10 mmol/l
Fast Red TR 6 mmol/l
3. Reagent (R3)
Sodium tartrate 400 mmol/l
Samples
Serum free of hemolysis. NO plasma can be used.

PROCEDURE

Preparation of working reagent

Dissolve one vial of reagent (R2) in an appropriate volume of buffer solution (R1).
Stability of working reagent
20-25°C: 6 hours
2-8 °C: 2 days
Light sensitive, store protected from light!
If the absorbance of working reagent is higher than 0.7 at 405 nm the reagent can not be used.

Assay conditions

Wavelength: 405 nm
Temperature: 37 °C
Cuvette: 1 cm pathway
Method: kinetic (increasing)
Pipette into cuvette

	Total	Tartrate resistant
Working reagent	1.0 ml	1.0 ml
Tartrate (R3)		80 µl
Sample or standard	100 µl	100 µl

Mix and incubate for 5 minutes, then read the absorbance in every minute for 3 minutes. Calculate the change of absorbance per minute (ΔA/min).

Calibration: (37 °C, Hillman's colorimetric method)
S1: Distilled water
S2: Randox Calibration Serum Level I
S3: Randox Calibration Serum Level II
Calibration frequency
Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Calculation using factor

Total acid phosphatase activity (U/l) = (ΔA/min x 750)
Total acid phosphatase activity (µkat/l) = (ΔA/min x 12,5)
Prostatic acid phosphatase activity (U/l) =
(ΔA/min(total)–ΔA/min(tartrate resistant))x 750
Prostatic acid phosphatase activity (µkat/l) =
(ΔA/min(total)–ΔA/min(tartrate resistant))x 12,5

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer.

Linearity

The test is linear up to 150 U/l (2,5 µkat/l) acid phosphatase activity.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 0.75 U/l (0,0125 µkat/l) Total acid phosphatase activity at 405 nm.

Precision

Total acid phosphatase

	Reproducibility		
	Average activity (U/l)	SD	CV%
Sample I.	23.4	0.320	1.36
Sample II.	37.5	0.283	0.76

Correlation

Comparative studies were done to compare our reagent with another commercial acid phosphatase assay.
The results from these studies are detailed below.
Correlation coefficient: r=0.9938
Linear regression: y (U/l)= 1.038x – 0.364
(x= other commercial reagent y= own reagent).

Specificity

Bilirubin 128.3µmol/l (7,5mg/dl), lipid 10,0 mg/dl, glucose 55.5 mmol/l (1000mg/dl), and ascorbic acid 2.84 mmol/l (0,05mg/dl) don't interfere with the assay up to the given levels.

Note

Do not pipette reagents by mouth.
Hemolytic as well as icteric sera interfere with the test. Test should be performed as soon as possible after blood sampling. 20 µl acetate buffer solution (5 mol/l) should be added to every ml of serum if samples have to be stored.
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use
Use by (last day of the month)
Temperature limitation
LOT Batch Code
REF Code

Bibliography

Hillmann, G.J.: Clin. Chem. Clin. Biochem. 9, 273 (1971).