Precautions in Use

In addition to the possible risks regarding the reactive components, product may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 1999/45/EC directives and modifications and amendments regarding classification, labelling and packaging of dangerous preparations (reagents) have been made accordingly. However, it is recommended that this product be handled carefully, that ingestion and contact with eyes, skin and mucous membranes be avoided and that laboratory reagents are used according to good laboratory practice.

Summary

Acid phosphatases are a group of enzymes that can be found in liver, spleen, erythrocytes, platelets, bone marrow and prostate gland. The ratio between total acid phosphatase and prostatic phosphatase in healthy males is about 1:1. Activity of total acid phosphatase increases in case of Paget’s disease, hyperparathyroidism, bone cancer, Gaucher’s disease, Niemann-Pick disease and myelocytic leukemia. Prostatic acid phosphatase levels increase in case of prostatic cancer.

Principle

Acid Phosphatase catalyses the hydrolysis, in acid medium, of 1-naphthylphosphate (1-NP) into 1-naphthol and phosphate. 1-naphthol reacts with diazo-2-chloro-5-toluene (Fast Red TR salt), forming an azo dye compound which absorbance increase is proportional to the total acid phosphatase activity (ACP). Prostatic acid phosphatase (ACP-P) is inhibited by tartrate and is detected with an indirect method by subtraction between ACP and non-prostatic acid phosphatase (ACP-NPP).

Reagents

Reagents, stored at 2-8 °C in unopened vials, are stable up to expiry date indicated on the package. Reagents must be limpid; do not use if turbid.

Components of the kit and initial concentration of reactive components:

- REAGENT 1a  (powder)
- REAGENT 1b  (powder)
- Tartrate REAGENT  (powder)
- Small Spoon  
- REAGENT 2  (acetic acid 0.1 mol/L)

Concentrations referred to Solution R1 (ACP) - see preparation of reagent solutions: citrate buffer 0.1 mol/L pH 5.4, 1-NP 10 mmol/L, Fast Red TR salt > 1.0 mmol/L

Concentrations referred to Solution R1 (ACP-NPP) - see preparation of reagent solutions: citrate buffer 0.1 mol/L pH 5.4, 1-NP 10 mmol/L, Fast Red TR salt > 1.0 mmol/L, sodium tartrate 0.18 mol/L

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

Notes and Limitations

Reagents Peculiar Information:

1) It is recommended to carry out the Prostatic Acid Phosphatase test only on samples with Total Acid Phosphatase activity > 3.5 U/L as it is possible to obtain not reliable results with a Total Acid Phosphatase activity lower than the above-mentioned limit.

2) A slight pink coloration of Solutions R1 does not affect the reagents performance.

Preparation of Reagent Solutions

Solution R1 (ACP): dissolve the contents of one bottle of REAGENT 1b with the contents of one bottle of REAGENT 1a. Let stand for about 10 minutes and gently mix. Stability: 7 days at 2-8 °C after reconstitution, if contamination avoided and vial recapped immediately after use.

Solution R1 (ACP-NPP): add one small spoon contents (corresponding to 35-40 mg) of Tartrate REAGENT to 5 mL of Solution R1 (ACP). Gently mix until complete solution. Stability: 7 days at 2-8 °C if contamination avoided and vial recapped immediately after use.

Solution R1 (ACP): dissolve the contents of one bottle of REAGENT 1b with 3 mL of REAGENT 1a. Let stand for about 10 minutes and gently mix. Stability: 7 days at 2-8 °C after reconstitution, if contamination avoided and vial recapped immediately after use.

Solution R1 (ACP-NPP): add one small spoon contents (corresponding to 35-40 mg) of Tartrate REAGENT to 1 mL of Solution R1 (ACP). Gently mix until complete solution. Stability: 7 days at 2-8 °C if contamination avoided and vial recapped immediately after use.

Calibration

For calibration, use only the following materials:

Clin Chem Cal  REF 16550  4x3mL  Lyophilized calibration serum. For use, follow the instructions contained in the kit.

Quality Control

To verify test accuracy use the following control materials at different levels of analyte:

Clin Chem Control 1  REF 16150  6x5mL
Clin Chem Control 2  REF 16250  6x5mL  Lyophilized control sera. For use, follow the instructions contained in the kit.
SAMPLE
Fresh serum. Do not use haemolized, icteric or lipemic samples. Collect samples in accordance with the NCCLS procedure reported in bibliography 1). Since the sample activity decreases of about 50% within an hour at room temperature, acidify the specimen immediately after collection by adding 20 µL of REAGENT 2 for each mL of fresh serum. Stability of acidified sample: 5 days at 2-8 °C.

INSTRUMENTATION AND MATERIALS
REQUIRED BUT NOT PROVIDED
• Usual laboratory equipment
• Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE
Wavelength: 405 nm
Pathlength: 1 cm
Temperature: 37 °C
Sample/Solution R1 (ACP): 1/10
Sample/Solution R1 (ACP-NPP): 1/10
Reaction: kinetic (increase)
Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

Put into cuvette:
ACP
ACP-NPP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solution R1 (ACP)</th>
<th>Solution R1 (ACP-NPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
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</tbody>
</table>

Mix and incubate for 4 minutes at working temperature. Read the absorbance after 1 minute and repeat the absorbance readings after exactly 1 and 2 minutes. Calculate the mean of ΔA/min found for both ACP and ACP-NPP.

CALCULATION
ACP: \( \Delta A/\text{min} \times F = \text{ACP activity in U/L} \)
ACP-NPP: \( \Delta A/\text{min} \times F = \text{ACP-NPP activity in U/L} \)
ACP-P: ACP - ACP-NPP = ACP-P (prostatic acid phosphatase) activity in U/L

This F factor is valid only for the above-mentioned working conditions (wavelength, sample volume, final reaction volume and pathlength).

REFERENCE VALUES
Serum
ACP: ≤ 4.7 U/L
ACP-P: ≤ 1.6 U/L

It is recommended that each laboratory establish its own expected range.

PERFORMANCES (determined on automatic analyzer)

Interferences (ACP/ACP-NPP): the test is not affected by the presence of bilirubin up to 20 mg/dL, hemoglobin up to 0.15 g/dL, and triglycerides up to 400 mg/dL.

Measuring range (ACP/ACP-NPP): 0.4 - 75 U/L. Samples with concentration higher than 75 U/L must be diluted 1:10 with normal saline and result multiplied by 10.

Intra-Assay Precision: it was determined on 20 replicates of each control (3 levels - L1/L2/L3). Results were as follows:
ACP - L1: average 6.7 U/L, SD 0.26, CV% 3.87 / L2: average 13.8 U/L, SD 0.37, CV% 2.70 / L3: average 28.0 U/L, SD 0.64, CV% 2.30.
ACP-NPP - L1: average 2.8 U/L, SD 0.19, CV% 6.73 / L2: average 3.9 U/L, SD 0.18, CV% 4.50 / L3: average 6.0 U/L, SD 0.23, CV% 3.86.

Inter-Assay Precision: it was determined for 10 days on 2 replicates of each control (3 levels - L1/L2/L3). Results were as follows:
ACP (Within Run) - L1: average 6.64 U/L, SD 0.26, CV% 3.94 / L2: average 13.84 U/L, SD 0.36, CV% 2.62 / L3: average 28.10 U/L, SD 0.61, CV% 2.16.
ACP (Run to Run) - L1: average 6.64 U/L, SD 0.27, CV% 4.06 / L2: average 13.84 U/L, SD 0.37, CV% 2.64 / L3: average 28.10 U/L, SD 0.62, CV% 2.22.
ACP-NPP (Within Run) - L1: average 2.93 U/L, SD 0.18, CV% 6.20 / L2: average 3.90 U/L, SD 0.17, CV% 4.41 / L3: average 5.96 U/L, SD 0.22, CV% 3.64.
ACP-NPP (Run to Run) - L1: average 2.93 U/L, SD 0.17, CV% 5.88 / L2: average 3.90 U/L, SD 0.19, CV% 4.95 / L3: average 5.96 U/L, SD 0.23, CV% 3.82.

Sensitivity (ACP/ACP-NPP): 0.4 U/L. Sensitivity was calculated on 10 replicates of normal saline and reported as the “mean zero value + 3 SD”.

Accuracy: this test (y) was compared with a commercially available method (x). Results were as follows:
ACP: N = 60, r = 0.99705, y = 1.0162 x + 0.10151
ACP-NPP: N = 60, r = 0.99788, y = 1.1325 x - 0.098874

WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.