ACID PHOSPHATASE

PRINCIPLE

The method is based on the hydrolysis of α-naphthyl phosphate at pH 5.0 by acid phosphatase (ACP) to produce α-naphthol and inorganic phosphate. The pentanedioil acts as a phosphate acceptor increasing the reaction sensitivity. The α-naphthol reacts with Fast Red TR, to produce a coloured complex directly proportional to the activity of the ACP in the sample.

\[
\text{ACP} + \text{α-Naphthyl phosphate} + \text{H}_2\text{O} \rightarrow \text{α-Naphthol} + \text{P}_i
\]

The sample tested in the presence of L-tartrate inhibits the prostatic acid phosphatase of the total ACP activity.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Citrate buffer</td>
<td>Sodium citrate 110 mmol/L, 1.5-pentanedio 220 mmol/L, pH 5.2.</td>
</tr>
<tr>
<td>R2</td>
<td>Citrate/Tartrate buffer</td>
<td>Sodium citrate 110 mmol/L, 1.5-pentanedio 220 mmol/L, L-tartrate 110 mmol/L, pH 5.2.</td>
</tr>
<tr>
<td>R3</td>
<td>ACP sustrate</td>
<td>Powder, α-Naphthyl phosphate 12.5 mmol/L, Fast Red TR 1.25 mmol/L, after reconstitution.</td>
</tr>
<tr>
<td>R4</td>
<td>Stabilizer</td>
<td>Acetate buffer 5 M/L, pH 5.2.</td>
</tr>
</tbody>
</table>

STORAGE AND STABILITY

Store at 2-8°C.

All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expirion date. Store the vials tightly closed, protected from light and prevented from contaminants during the use.

REAGENT PREPARATION

Working reagent. Add 10 mL of R1 (total ACP) or 10 mL of R2 (non-prostatic ACP) into a vial of R3. Cap and swirl gently until complete solution. Do not shake. The reagent is stable for 10 days at 2-8°C.

Sample or control. Add 1.0 mL of working reagent R1 and 1.0 mL of working reagent R2 into labelled cuvettes.

CALCULATIONS

A. Total Acid Phosphatase

\[
\text{U/L} = \Delta A \text{ min} \times 853
\]

B. Non-Prostatic Acid Phosphatase

\[
\text{U/L} = \Delta A \text{ min} \times 853
\]

C. Prostatic Acid Phosphatase

\[
A \text{ (U/L)} = B \text{ (U/L)} - \text{Prostatic Acid Phosphatase}
\]

Examples with ΔA/min exceeding 0.170 at 450 nm should be diluted 1:3 with saline and assayed again. Multiply the results by 3.

If results are to be expressed as SI units apply:

\[
\text{U/L} \times 16.67 = \mu\text{kat/L}
\]
REFERENCE VALUES

<table>
<thead>
<tr>
<th>Reaction temperature</th>
<th>Total ACP, up to</th>
<th>Prostatic ACP, up to</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>6.6 U/L (110 nkat/L)</td>
<td>3.5 U/L (108 nkat/L)</td>
</tr>
<tr>
<td>30°C</td>
<td>7.0 U/L (278 nkat/L)</td>
<td>2.6 U/L (43 nkat/L)</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

<table>
<thead>
<tr>
<th>REF 1980005</th>
<th>HUMAN MULTISERA NORMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline level of ACP. Assayed.</td>
<td></td>
</tr>
</tbody>
</table>

If the values are found outside of the defined range, check the instrument, reagents and procedure. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Determination of acid phosphatase activity in serum is directed toward the prostatic enzyme with the intent of detecting carcinoma of the prostate.

Elevations of the activity are found in the sera of about 60% of men with prostatic cancer with metastases. Slight elevations in total enzyme are observed in cases of thromboembolic phenomena, multiple myeloma, thrombocytopenia and liver disease.

Moderate elevations in total acid phosphatase activity often occur in Paget’s disease, in hyperparathyroidism with skeletal involvement, and in the presence of malignant invasion of the bones by cancers. The serum activity in these cases is not inhibited by tartrate. The only non-bone condition in which elevated activities of tartrate-resistant osteoclast-type acid phosphatase are found in serum is Gaucher’s disease.

ANALYTICAL PERFORMANCE

- Detection Limit : 2.74 U/L

- Linearity : Up to 150 U/L

- Precision:

<table>
<thead>
<tr>
<th>U/L</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>28.2</td>
<td>28.2</td>
</tr>
<tr>
<td>SD</td>
<td>0.40</td>
<td>1.41</td>
</tr>
<tr>
<td>CV%</td>
<td>1.43</td>
<td>4.98</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

- Sensitivity : 1.3 mA / U/L. Acid phosphatase.

- Correlation: This assay (y) was compared with a similar commercial method (x). The results were:

\[ N = 50 \quad r = 0.987 \quad y = 1.078 x - 2.166 \]

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.

2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES