ACID PHOSPHATASE REAGENT SET (KINETIC)

For the quantitative determination of acid phosphatase in serum.

SUMMARY AND EXPLANATION

Non-specific acid phosphatase activity is widely distributed throughout the living world. This enzyme secreted by the human prostate gland has attracted most attention, because of its clinical importance, and extensive characterization and structural studies have have now been carried out on it. Since acid phosphatase is also produced in other tissues, the prostate isoenzyme must be distinguished from the non-prostatic for accurate diagnosis. Elevated levels of non-prostatic acid phosphatase have been observed in patients with Paget's disease, hyperparathyroidism with skeletal involvement, and in cancers which have invaded the bones.1,2

Numerous phosphate compounds have been proposed as substrates for measuring acid phosphatase activity, such as phenylphosphate, p-nitrophenylphosphate, thymolphthalein phosphate. α-Naphthylphosphate was proposed by Babson et al3 as a specific substrate for prostatic acid phosphatase. However, Amador et al4 demonstrated that this compound can be hydrolyzed by enzymes derived from other tissues. Hillman5 proposed a method in 1971 that included diazo dye that absorbed strongly at 405nm. L-Tartrate was used in a study for a specific inhibitor of prostatic acid phosphatase to establish differentially the amount of prostatic isoenzyme.6 The above kinetic method is specific, fast, simple and can easily be adopted to automated instrumentation.

PRINCIPLE
α-naphthylphosphate + H₂O ----→ a-naphthol + inorganic phosphorus
α-naphthol + Fast Red TR----→ Diazo Dye (Chromophore)

The α-naphthol released from the substrate α-naphthylphosphate by acid phosphatase is coupled with Fast Red TR that formed a diazo dye that absorbed strongly at 405nm. L-Tartrate was used as a specific inhibitor of prostatic acid phosphatase to establish differentially the amount of prostatic isoenzyme. The α-naphthol can be quantitated photometrically because the coupling reaction is instantaneous.

L-Tartrate inhibits prostatic acid phosphatase but does not interfere with the reaction mechanism. Therefore, if testing is performed in the presence and in the absence of L-Tartrate, the difference between the results of the two assays is the level of prostatic acid phosphatase in the serum.

REAGENTS: For in vitro diagnostic use.
1. Acid phosphatase reagent (Concentrations refer to reconstituted reagent): α-naphthylphosphate 3mM, Citric Acid 20mM, Fast Red TR 1mM, Sodium Citrate 60mM, pH 5.3±0.1.
2. L-Tartrate Reagent (Concentrations refer to reconstituted reagent): Sodium L-Tartrate 2M, Citric Acid 70mM, Sodium Citrate 10mM, pH 5.3±0.1.
3. Acetate Buffer: 5M, pH 5.0.

REAGENT PREPARATION:
1. Reconstitute acid phosphatase with the volume of distilled water stated on the label. Swirl to dissolve. 2. Reconstitute L-Tartrate Reagent with 5.0 ml distilled water. Warm reagent to aid in dissolution, if necessary. 3. Acetate Buffer is ready to use.

REAGENT STORAGE AND STABILITY:
1. Unopened vials are stable until stated expiration date on the vial label when stored refrigerated (2°-8°C).
2. The reconstituted acid phosphatase reagent is stable for one day at room temperature (22°-28°C) and for seven days when stored refrigerated at 2°-8°C.
3. The reconstituted L-Tartrate Reagent is stable refrigerated 2°-8°C until the expiration date listed on the vial label. If crystallization of component occurs, warm at moderate temperature (40°-50°C) to redissolve reagent.
4. Acetate Buffer Reagent is stable refrigerated 2°-8°C until the expiration date listed on the vial label.

REAGENT DETERIORATION
The reagent should not be used if:
1. The reconstituted acid phosphatase reagent, without serum added, has an absorbance greater than 0.4 when measured at 405nm against water.
2. The L-Tartrate Reagent is precipitated. Apply heat (40°-50°C) to redissolve reagent.

SPECIMEN COLLECTION AND STORAGE
1. Use only clear, unhemolyzed serum.
2. Serum must be separated from clot within two hours after collection.
3. Acid Phosphatase activity is extremely labile at room temperature. Stabilization of the enzyme can only be achieved by acidifying with the Acetate Buffer provided. Add 20μL (0.02ml) of buffer per 1.0ml of serum. Mix. Treated serum samples will remain stable for seven days when kept refrigerated at 2°-8°C.
4. Do not use plasma. Some coagulants inhibit acid phosphatase activity and/or cause turbidity.

INTERFERENCES
1. High levels of bilirubin (Icteric Samples) reportedly inhibit acid phosphatase activity determined by this procedure.9
2. A number of drugs and substances affect Acid Phosphatase activity. Young, et al10 has published a comprehensive list.

MATERIALS REQUIRED BUT NOT SUPPLIED
1. Test tubes/rack.
2. Accurate dispensing devices.
3. Distilled/deionized water.
4. Timer.
5. Spectrophotometer capable of reading at 405nm.
6. Temperature must be closely controlled during assay. A temperature controlled (37°C) spectrophotometer cuvette should be used.

PROCEDURE: (AUTOMATED)
Refer to specific instrument application instructions.

PROCEDURE (MANUAL)
NOTE: Stabilize acid phosphatase immediately after separation of the serum from the clot by adding 20μL (0.02ml) of Acetate Buffer per 1.0ml of serum. Mix and store in refrigerator until assay is ready to be performed.

A. TOTAL ACID PHOSPHATASE
1. Reconstitute reagent according to instructions.
2. Label tubes. "CONTROL", "PATIENT", etc.
3. Pipette 1.0ml of reagent into all tubes.
4. Zero spectrophotometer with water at 405nm. Set cuvette temperature at 37°C.
5. Perform the following steps individually on each sample.
   a). Add 100μl (0.10ml) of sample to respective tube and allow to incubate at 37°C for five minutes.
   b). After incubation, read and record absorbance every minute for five minutes to determine ΔA/Minute.
   c). Repeat procedure for each sample.
6. Values (UL) are obtained by multiplying ΔA/Minute by the factor. See "Calculations".

B. NON-PROSTATIC ACID PHOSPHATASE
1. Add 1.0ml of reagent to appropriately labeled tube.
2. Add 10μL (0.01ml) of L-Tartrate Reagent and mix.
3. Zero spectrophotometer with water at 405nm. Set cuvette temperature to 37°C.
4. Perform the following steps individually on each sample.
   a). Add 100μl (0.10ml) of sample, mix and incubate at 30°C for five minutes.
   b). After incubation, read and record absorbance every minute for five minutes to determine ΔA/Minute.
   c). Repeat procedure for each sample.
6. Values (UL) are obtained by multiplying ΔA/Minute by the factor. See "Calculations".

C. PROSTATIC ACID PHOSPHATASE
The value is obtained by subtracting the results of the non-prostatic acid phosphatase assay (B) from the total acid phosphatase assay (A).

QUALITY CONTROL
1. The integrity of the reaction should be monitored by use of a normal and abnormal control serum with known acid phosphatase values.
2. Acid Phosphatase in control sera is more labile than in fresh sera. Add 20μL (0.02ml) of acetate buffer per 1.0ml of water used to reconstitute the control sera.

CALCULATIONS
One International Unit is defined as the amount of enzyme catalyzes the transformation of one micromole of substrate per minute under defined conditions.
A. Total Acid Phosphatase Calculation.

\[
\Delta A/\text{MIN} \times 1000 \times 1.1 = \Delta A/\text{MIN} \times 853 \times 12.9 \times 1.0 \times 0.1
\]

B. Non-prostatic Acid Phosphatase Calculation

\[
\Delta A/\text{MIN} \times 1000 \times 1.11 = \Delta A/\text{MIN} \times 860 \times 12.9 \times 1.0 \times 0.1
\]

Where:
- 1000 = Conversions of u/mL to U/L
- 1.1 = Total reaction volume (total A.P)
- 1.11 = Total reaction volume (non-Prost. A.P.)
- 12.9 = Molar absorptivity of α-naphthol-Fast Red Complex at 405nm
- 1.0 = Light path in cm.
- 0.1 = Sample volume (ml)

SAMPLE CALCULATION
AA/MIN. total acid phosphatase = 0.01
AA/MIN. Non-Prostatic acid phosphatase = 0.009
Total acid phosphatase: 0.01 x 853 = 8.5 U/L
Non-Prostatic acid phosphatase: 0.009 x 860 = 7.7 U/L
Prostatic Acid phosphatase: 8.5 - 7.7 = 0.8 U/L

LIMITATIONS
Samples with values above 60 U/L at 37°C should be diluted 1:9 with normal saline, re-run, and the final results multiplied by 10.

EXPECTED VALUE
Total Acid Phosphatase: 0.9 U/L
Prostatic Acid Phosphatase: 0.3 U/L

Values were taken from literature, these values are referred to adults, both male and females. It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE
1. Linearity = 60 U/L
2. Comparison = A study performed using the method with a commercial reagent with a similar formulation yielded the following: N = 22.

<table>
<thead>
<tr>
<th>Correlation Coefficient</th>
<th>Total</th>
<th>Prostatic</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.97</td>
<td>0.98</td>
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<tr>
<td>Regression Equation</td>
<td>Y=0.96X+0.38</td>
<td>Y=0.97X-0.23</td>
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</table>

3. Precision

<table>
<thead>
<tr>
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<th>Within Run (N=20)</th>
<th>Run to Run (N=15)</th>
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<tbody>
<tr>
<td></td>
<td>Total Acid Phosphatase</td>
<td>Total Acid Phosphatase</td>
</tr>
<tr>
<td>Mean (u/L)</td>
<td>10.2</td>
<td>10.3</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>C.V.%</td>
<td>11.7</td>
<td>7.4</td>
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REFERENCES

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