

Intended Use

For the quantitative determination of acid phosphatase in serum.

Clinical Significance

Large elevations of prostatic acid phosphatase are found in cases of metastasized prostatic cancer. Since acid phosphatase is also produced in other tissues, the prostatic 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.⁷

Method History

Phosphate compounds proposed throughout the years as substrates for measuring acid phosphatase activity included Phenylphosphate, α -Glycerolphosphate, p -Nitrophenylphosphate and Thymolphthalein Phosphate. Most of the above substrates were either insensitive to the small increases in prostatic acid phosphatase activity, or were too sensitive to non-prostatic acid phosphatase in the serum. Roy et al¹ proposed a method using Sodium Thymolphthalein Monophosphate as a specific substrate for prostatic acid Phosphatase in 1971. A modification by Ewen and Spitzer² in 1976 improved the sensitivity of the Roy method. Even though the modified procedure has found wide acceptance, it suffers from being a long and tedious procedure as well as not being totally specific for the prostatic acid phosphatase; also measuring erythrocyte and platelet acid phosphatase.

In 1959, Babson et al³ proposed Alpha-naphthylphosphate as a specific substrate for prostatic acid phosphatase. The specificity was disputed by Amador⁴ in 1969.

Hilman⁵ proposed a method in 1971 that included diazotized 2-amino-5-chlorotoluene (Fast Red TR) that formed a diazo dye that absorbed strongly at 405 nm. L-Tartrate was used as 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 be easily adapted to automated instrumentation.

Principle

Acid Phos.

α -naphthylphosphate + H₂O -----> α -naphthol + I. Phos.

α -naphthol + Fast Red TR -----> DiazoDye (Chromophore)

α -naphthylphosphate is hydrolyzed by serum acid phosphatase to α -naphthol and inorganic phosphate. The rate of hydrolysis is proportional to the enzyme activity present. The α -naphthol produced is coupled with Fast Red TR to produce a colored complex which absorbs light at 405 nm. The reaction 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

1. Acid Phosphatase reagent (Concentrations refer to recon. reagent): α -naphthylphosphate 3mM, Fast Red TR 1mM, Citric Acid 20mM, Sodium Citrate 60mM, pH 5.3 \pm 0.1.
2. L-Tartrate Reagent (Concentrations refer to recon. reagent): Sodium L-Tartrate 2M, Citric Acid 70mM, Sodium Citrate 10mM, pH 5.3 \pm 0.1.
3. Acetate Buffer: 5M, pH 5.0.

Reagent Preparation

1. Reconstitute acid phosphatase reagent with 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 dissolution, if necessary.
3. Acetate buffer is ready to use.

Reagent Storage

1. Unopened vials are stable until stated expiration date on vial label when stored refrigerated (2-8°C).
2. The reconstituted acid phosphatase reagent is stable for 24 hours at room temperature (22-28°C) and for 14 days when stored refrigerated (2-8°C).
3. The reconstituted L-Tartrate Reagent is stable refrigerated (2-8°C), until expiration date on vial label. If crystallization occurs, warm at moderate temperatures (40-50°C) until dissolved.
4. Acetate Buffer solution 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 0.300 when measured at 405 nm against water.
2. The L-Tartrate Reagent is precipitated. Apply heat (40-50°C) to re-dissolve reagent.

Precautions

This reagent is for *in vitro* diagnostic use only.

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 Acetate buffer provided. **Add 20ul (0.02ml) of buffer per 1.0ml of serum. Mix.** Treated serum samples will remain stable for 7 days when kept refrigerated at 2-8°C.⁸
4. Do not use plasma. Some anticoagulants inhibit acid phosphatase activity and/or cause turbidity.⁹

Interferences

1. High levels of bilirubin (icteric samples) reportedly inhibit acid phosphatase activity determined by this procedure.¹⁰
2. A number of drugs and substances affect Acid Phosphatase activity. Young, et al¹¹ has published a comprehensive list.

Materials Provided

1. Acid Phosphatase Reagent.
2. L-Tartrate Reagent.
3. Acetate Buffer.

Materials Required but not Provided

1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 405 nm.
5. Distilled/Deionized water.
6. Temperature must be closely controlled during assay. A temperature controlled (30 or 37°C) cuvette should be used.

Procedure (Automated)

Refer to specific instrument application instructions.

Acid Phosphatase Reagent Set

Procedure (Manual)

Note: Stabilize acid phosphatase immediately after separation of the serum from the clot by adding 20ul (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 to 30 or 37°C.
5. Add 100ul (0.100 ml) of sample to respective tube and allow to incubate for five minutes.
6. After incubation, read and record absorbance every minute for five minutes to determine $\Delta A/\text{minute}$.
7. Repeat procedure for each sample.
8. Values (u/L) are obtained by multiplying the $\Delta A/\text{minute}$ by the factor. See "Calculation".

B. Non-Prostatic Acid Phosphatase

1. Add 1.0ml of reagent to appropriately labeled tube.
2. Add 10ul (0.010 ml) of L-Tartrate reagent and mix.
3. Zero spectrophotometer with water at 405nm. Set cuvette temperature to 30 or 37°C.
4. Add 100ul (0.100 ml) of sample, mix and incubate for five minutes.
5. After incubation, read and record absorbance every minute for five minutes to determine $\Delta A/\text{minute}$.
6. Values (u/L) are obtained by multiplying $\Delta A/\text{minute}$ by the factor. See "Calculation".

C. Prostatic Acid Phosphatase

1. The value is obtained by subtracting the result of the non-prostatic acid phosphatase assay (B) from the total acid phosphatase assay (A).

Limitations

Samples with values above 35u/L at 30°C, or above 40u/L at 37°C, should be diluted 1:9 with saline, re-run, and the final result multiplied by 10.

Calculation

One international unit is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined conditions.

A. Total Acid Phosphatase Calculation.

$$\frac{\Delta A/\text{Min} \times 10^6 \times 1.1}{12.9 \times 10^3 \times 1.0 \times 0.1} = u/L = \Delta A/\text{Min} \times 853$$

B. Non-prostatic Acid Phosphatase Calculation.

$$\frac{\Delta A/\text{Min} \times 10^6 \times 1.11}{12.9 \times 10^3 \times 1.0 \times 0.1} = u/L = \Delta A/\text{Min} \times 860$$

Where:

- 10^6 = Conversion of moles to millimoles
- 1.1 = Total reaction volume (total A.P.)
- 1.11 = Total reaction volume (Non-Prost. A.P.)
- 12.9×10^3 = Molar absorptivity of α -naphthol Fast Red TR Complex at 405nm.
- 1.0 = Light path in cm.
- 0.1 = Sample volume (ml).

Sample Calculations:

$$\Delta A/\text{Min. total acid phosphatase} = 0.010$$

$$\Delta A/\text{Min. Non-Prostatic acid phosphatase} = .009$$

$$\text{Total acid phosphatase: } 0.010 \times 853 = 8.5 \text{ u/L}$$

$$\text{Non-Prostatic acid phosphatase: } .009 \times 860 = 7.7 \text{ u/L}$$

$$\text{Prostatic Acid Phosphatase: } 8.5 - 7.7 = 0.8 \text{ u/L}$$

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 20ul (0.02 ml) of acetate buffer per 1.0ml of water used to reconstitute the control sera.

Expected Values

Total Acid Phosphatase: 0-9u/L at 30°C, 2.5-11.7u/L at 37°C

Prostatic Acid Phosphatase: 0-3u/L at 30°C, 0.2-3.5u/L at 37°C

Values were taken from literature.¹² It is strongly recommended that each laboratory establish its own normal range.

Performance

1. Linearity: 35 u/L at 30°C, 40u/L at 37°C
2. Comparison: A study performed using this method with a commercial reagent with a similar formulation yielded the following:

N= 26	Total	Prostatic
Correlation Coefficient	0.998	0.994
Regression Equation	$y=0.97x-0.40$	$y=0.97x-0.25$

3. Precision:

Within Run (N=15)			Run to Run (N=15)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
8.7	0.14	1.6 (Total)	3.7	0.28	7.6
33.3	0.29	0.9 (Total)	7.8	0.18	2.3
7.2	0.57	7.9 (Prostatic)	32.7	0.36	1.1
29.4	0.67	2.3 (Prostatic)			

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