ACID PHOSPHATASE
Insert

Ref.: 39

Intended use . System for acid phosphatase determination in blood sample by kinetic method of fixed time and measurement of end point.

Test principle . Acid phosphatase of serum acts on the monophosphate thymolphthalein substrate. The addiction of alkali inhibits the enzymatic action and converts the released thymolphthalein in it blue form that is measured by colorimetry. The final product of reaction consists of a mixture of blue and the substrate color.

Summary . During the selection of a substrate for determination of acid phosphatase, Labtest have focused on monophosphate thymolphthalein research, that posses more specificity to prostatic isoenzymes than the phenyl phosphate and p-nitro phenyl phosphate.

Thymolphthalein specificity to the prostatic fraction is not absolute. Therefore, there is a little activity in serum of healthy women.

This method provides lower values than the obtained using p-nitrophenyl or phenyl phosphate substrate that are also hydrolyzed by phosphatase from hematic and platelets origin.

The technical procedure performed in 30 minutes is extremely simple and has a proportional response to the enzymatic activity up to 200 folds the values of reference with only one colorimetric measurement.
In spite of measuring the total acid phosphatase, increased results may be considered as being correspondent to prostatic fraction because the sensibility of the substrate to other fractions is considerably decreased and the values found to the non-prostatic phosphatase activity are so reduced that are not clinically significant.

Methodology . Roy modified

Reagents

1. R 1 **- Substrate - Store at 2 - 8 ºC.**
Reagent label bears expiration date. After dissolved, it contains monophosphate thymolphthalein (1.5 mmol/L).

2. R 2 **- Color Reagent - Store at 15 - 25 ºC.**
Reagent label bears expiration date. Sodium carbonate (50 mmol/L) and sodium hydroxide (50 mmol/L).

3. CAL **- Standard 3.0 U/L - Store at 2 - 8 ºC.**
Reagent label bears expiration date. In order to avoid evaporation of the Standard, keep the bottle tightly closed. Thymolphthalein (0.09 mmol/L).

4. R 4 **- Buffer - Store at 15 - 25 ºC.**
Buffer (100 mol/L), pH 5.95 and sodium azide (7.7 mmol/L).

Precautions and warnings

For in vitro diagnostic use.

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

Buffer contains sodium azide, what is toxic, as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Cares regarding reaction time, temperature of incubation and pipetting are extremely important in order to obtain correct results. The difference of one minute in the incubation time induces an error of 3.3% in the results.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.

Deterioration . Microbial or chemical contamination may decrease reagents stability.

Specimen collection and preparation

Use serum or plasma (heparine). The enzymatic activity is sensible to temperature and pH effects. Therefore, separate the serum or plasma within 30 minutes after collecting and add 0.01 mL of acetic acid 20% (v/v) for each 1.0 mL of sample. The acidified sample is reportedly stable in for about 2 days at 2 - 8 ºC and 1 week at -10 ºC.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Bilirubin up to 5 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 750 mg/dL do not interfere significantly.

Bilirubin values over 5 mg/dL and triglycerides values over 750 mg/dL provide false increased results.

Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 570 - 610 nm.
3. Pipettes to measure reagents and samples.
4. Timer.

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Manual procedure

See notes 1, 2 and 3.

Preparing the working reagent . Dissolving the substrate: Transfer all the contents of Buffer (20 mL) to substrate bottle and mix gently by inversion until complete dissolution. It is stable 6 months at 2 - 8 ºC.

Set up three tubes and proceed as follows:

	Control	Unknown	Standard
Substrate (nº 1)	0.5 mL	0.5 mL	----
Distilled or deionized water	----	----	0.5 mL
Standard (nº 3)	----	----	0.1 mL

Mix and incubate in a water bath at 37 ºC during 2 minutes.

Sample	----	0.1 mL	----

Mix and incubate in a water bath at 37 ºC during 30 minutes.

Color reagent (nº 2)	2.0 mL	2.0 mL	2.0 mL
Sample	0.1 mL	----	----

Mix and measure the absorbance of the Control, Unknown and Standard against distilled water at 590 nm or orange filter (570 - 610). The color is stable during 120 minutes.
The suggested measurement procedure is appropriated to photometer of which the minimal volume of solution for reading is equal or lower than 2.5 mL. It should be done a verification of the necessity of volume adjustment for the photometer to be used. Sample and reagent volume may be modified proportionally without affecting the test performance and the calculation procedure. In case of volume reduction is important to observe the minimum volume needed to the photometric reading. Volume of sample lower than 0.01 mL is critical in manual applications and should be avoided because it increases the measurement imprecision.

Quality control . For quality control use Qualitrol Level 1 and Qualitrol Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Calculations

$$\text{Acid Phosphatase (U/L)} = \frac{A_{Unknown} - A_{Control}}{A_{Standard}} \times 3$$

Due the great reproductive results of the assays system, it is possible to use the factor method:

$$\text{Calibration factor} = 3 / A_{Standard}$$

$$\text{Acid Phosphatase (U/L)} = (A_{Unkown} - A_{Control}) \times \text{Factor}$$

Measurement/reportable range

Up to 110 U/L

If acid phosphatase activity exceeds 110 U/L, the unknown and control must be diluted with the Color Reagent. Multiply the result by the appropriate dilution factor. If afterwards the result is equal to or more than 110 U/L, repeat the measurement reducing to 10 minutes the incubation time after adding the sample. Multiply the result by 3.

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

0.15 to 0.56 U/L

Unit definition: a Unit is the amount of enzyme that yields, by hydrolysis, 1 μmol of thymolphthalein per minute, per liter of serum, in the test conditions.

Performance characteristics

Recovery studies . In two samples with acid phosphatase values of 0.6 and 1.5 U/L were added different quantities of the enzyme. Subsequent analyses provided recoveries ranging from 96 to 106%. The mean proportional systematic error at 0.5 U/L was 0.005 U/L or 1.0%.

Method comparison . A group of 80 sera were assayed by the proposed method and a technique using p-nitrophenil phosphate. Serum acid phosphatase values ranged from 0.14 - 11.0 U/L. The comparisons yielded a correlation coefficient of 0.99 and regression equation was $y = 0.044 + 0.339x$. There is a positive correlation among both methods, observing a 56% systematic difference when the decision level is 0.5 U/L, what is explained by the difference of the substrates used and the methodology.

Imprecision - Within run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	0.64	0.02	3.4
Sample 2	20	1.52	0.02	1.1
Sample 3	20	2.71	0.02	0.6

Imprecision - Run-to-run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	0.58	0.04	7.4
Sample 2	20	1.46	0.05	3.2
Sample 3	20	2.69	0.03	1.1

Analytical sensitivity . Detection limit: 0.02 U/L. The detection limit represents the lowest measurable acid phosphatase activity that can be distinguished from zero. Using the standard absorbance as parameter, it was verified that the detection limit is 0.02 U/L, corresponding to a absorbance of 0.001.

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Matrix dilution effects . Two sample with values equal of 85 and 113 U/L were used to evaluate the system response on the reduction of incubation time. Recoveries were found a range of 96 and 112 %, using incubation time that varies from 10 to 20 minutes.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

3. Cares regarding reaction time, temperature of incubation and pipetting are extremely important in order to obtain correct results. The difference of one minute during incubation time of this measurement induces an error of 3.3% in the result.

4. It is suggested to consult "Young DS. Effects of Drugs on Clinical Laboratory Tests, 3rd Edition, Washington: AACC Press, 1990." in order to review physiopathological source and drugs interference in results and methodology.

References

1. Coleman CM, Stroje RC.: Clin Chim Acta 1966:13:401.
2. Bergmeyer HU. Methods of Enzymatic Analisys, 3 ed. Vol 4, Deerfiled Beach: Verlag Chemie, 1984:92-100.
3. Roy AV, Brower ME, Hayden JE. Clin Chem 1971;17:1093.
4. Tonks DB Quality Control in Clinical Laboratories, Warner Chilcot laboratories, Diagnostic Reagents Division, Scarborough, Canada, 1972.
5. Westgard JO, Groth T. Clin Chem 1981; 27:493-501.
6. Labtest: data on file.

Presentation

Product	Reference	Contents		
Acid Phosphatase	39	R 1	1 X 30 µmL	LYOPH
		R 2	1 X 100 mL	
		CAL	1 X 3 mL	
		R 4	1 X 20 mL	

Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

 **Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests	 **Risco biológico** Riesgo biológico Biological risk
 **Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	 **Marca CE** Marcado CE CE Mark
 **Material Calibrador** Material Calibrador Calibrator Material	 **Tóxico** Tóxico Poison
 **Material Calibrador** Material Calibrador Calibrator Material	 **Reagente** Reactivo Reagent
 **Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)	 **Fabricado por** Elaborado por Manufactured by
 **Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	 **Número do lote** Denominación de lote Batch code
 **Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	 **Controle** Control Control
 **Número do catálogo** Número de catálogo Catalog Number	 **Controle negativo** Control negativo Negative control
 **Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	 **Controle positivo** Control positivo Positive control
 **Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	 **Controle** Control Control
 **Liofilizado** Liofilizado Lyophilized	 **Corrosivo** Corrosivo Corrosive
 **Período após abertura** Período post-abertura Period after-opening	Ref.: 201112

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