CHOLINESTERASE Liquiform

Insert

Ref.: 139

Purpose . System for determining cholinesterase activity (pseudocholinesterase or cholinesterase II) in serum or plasma samples by kinetic reaction.

[Only for in vitro diagnostic use.]

Principle . The cholinesterase (CHE) catalyzes the hydrolysis of butyrylthiocholine, with formation of butyrate and thiocholine. The thiocholine reduces potassium ferricyanide, yellow color, into potassium ferrocyanide, which is practically colorless.

The decrease in absorbance at 405 nm as a result of the reduction of potassium ferricyanide is monitored photometrically, being directly proportional to the cholinesterase activity in the sample.

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{CHE}} \text{Butyrate} + \text{Thiocholine}$$

$$2\,\text{Thiocholine} + 2\,[Fe(CN)_6]^{3-} + 2\,OH^- \rightarrow \text{Dithiobis(choline)} + H_2O + 2\,[Fe(CN)_6]^{4-}$$

System features . The kinetic methodology for measuring cholinesterase activity was introduced by Dietz *et al*[1] and later modified by the *Deutsche Gesellschaft für Klinische Chemie (DGKC)*[2].

The system uses the butyrylthiocholine substrate, which has higher specificity for cholinesterase II, minimizing the interference of acetylcholinesterase (cholinesterase I) present in hemolyzed samples.

The reagents are ready to use, facilitating their application in semi-automatic and automatic systems, capable of performing measurements in kinetic mode at wavelength of 405 nm.

Methodology . Kinetic - DGKC

Reagents

1. R 1 **- Reagent 1 - Store at 2 - 8 ºC.**
Contains 90 mmol/L pyrophosphate buffer and potassium ferricyanide ≥2 mmol/L.

2. R 2 **- Reagent 2 - Store at 2 - 8 ºC.**
Contains butyrylthiocholine ≥15 mmol/L.

The unopened reagents, when stored under the specified conditions, are stable until the expiration date printed on label. Opened reagents must be handled in accordance with good laboratory practices to avoid contamination of microbial and chemical nature that can cause reduced stability.

Warnings and special cares

Usual safety care should be applied in the handling of reagents.

In automatic analyzers, the reagents are subject to contamination by other reagents or environmental air, which depend on the equipment feature and work conditions. These contaminations can result in reduced stability of the reagents or modifications in their performance.

Materials required and not provided

1. Water bath maintained at constant temperature of 37 ºC.
2. Photometer with thermostated cuvette at 37 ºC, capable of measuring with accuracy the absorbance at 405 nm.
3. Pipettes to measure samples and reagents.
4. Chronometer.
5. Calibrator - Calibra H Line - Ref 80, Labtest.

Sample

A Standard Operating Procedure (SOP) should be elaborated to establish appropriate procedures for collection, preparation and storage of the sample. We emphasize that errors originated from the sample can be much larger than the errors occurred during the analytical procedure.

Use serum or plasma (EDTA, Heparin). **Do not use fluoride-containing anticoagulants because they inhibit cholinesterase activity.** The enzyme activity remains stable in samples stored for 15 days at 2 - 8 ºC and 6 months at 20 ºC negative.

Since no known test can ensure that blood samples will not transmit infectious diseases, all samples should be deemed potentially infectious. Therefore, when handling them, follow the rules established for biosafety.

For disposal of reagents and biological material, we suggest applying the applicable local, state or federal environmental protection standards.

Interference

Bilirubin concentrations up to 20 mg/dL, hemoglobin up to 200 mg/dL, and triglycerides up to 1200 mg/dL do not produce significant interference.

To evaluate the approximate concentration of hemoglobin in hemolyzed samples, you can proceed as follows: Dilute 0.05 ml of sample in 2.0 mL of 150 mmol/L NaCl (0.85%) and measure the absorbance at 405 or 415 nm hitting the zero with distilled or deionized water.

$$\text{Hemoglobin (mg/dL)} \cong \text{Absorbance}_{405} \times 601$$
$$\text{Hemoglobin (mg/dL)} \cong \text{Absorbance}_{415} \times 467$$

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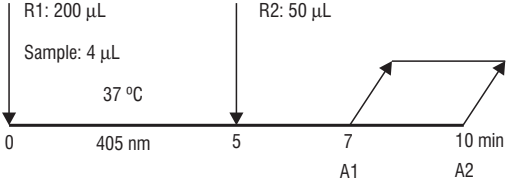
Procedure

Parameters for automated analyzers

Parameters	Application
Reaction Type	Kinetic
Reaction Direction	Decreasing
Primary λ	405 nm
Secondary λ	700 nm
Temperature	37 ºC
Calibration	2 points Point 0: Blank (Deionized water / Saline) Point 1: Calibrator 1
Calibration Model*	Linear
Sample Volume**	4 µL
R1 Volume**	200 µL
R2 Volume**	50 µL
Addition of R2	300 seconds after adding R1 + sample
Reading 1 (Abs 1)	120 seconds after adding R2
Reading 2 (Abs 2)	300 seconds after adding R2



*The definition of calibration model must be appropriate for each type of equipment. If in doubt, please contact the Labtest Customer Service.

**The sample and reagent volumes can be modified proportionately without affecting the test performance. In case of reduced volumes it is essential to observe the minimum volume required for the photometric measurement.

Manual procedure

Optimum reaction conditions:
Wavelength: 405 nm;
Thermostated cuvette at 37 ± 0.2 ºC with 1.0 cm thick solution;
Passband ≤2 nm;
Stray light ≤0.1.

Take two test tubes and proceed as follows:

Part 1

	Test	Calibrator
Sample	0.02 mL	----
Calibrator*	----	0.02 mL
Reagent 1	1.0 mL	1.0 mL

*It is advisable to use calibrator Calibra H - Labtest.

Mix and incubate in water bath at 37 ºC for 3 minutes. The water level in the bath should be higher than the level of reagent in test tubes.

Part 2
Add to the two tubes

	Test	Calibrator
Reagent 2	+ 0.25 mL	+ 0.25 mL

1. Mix and transfer immediately to a thermostated cuvette at 37 ± 0.2 ºC. Wait for 2 minutes.

2. Read the initial absorbance (A_1) at 405 nm simultaneously firing the chronometer. Repeat the reading after 3 minutes (A_2).

As with any measurement of enzyme activity, strict observation of time and incubation temperature is of great importance for the quality of the results.

To assess the linearity of the reaction, record the absorbance at 1 minute intervals and check whether the differences in absorbance are equivalent in every minute.

The procedures suggested are proper suitable for photometers with minimum reaction volume for measurement equal to or less than 1.0 mL. It must be checked the need for adjustment of the volume used in the photometer. The sample and reagent volumes can be modified proportionately without affecting the performance of the test and calculations remain unchanged. In the case of reduced volumes it is essential to observe the minimum volume required for photometric measurement. Sample volumes smaller than 0.010 mL are critical in manual applications and should be used with caution as they increase the inaccuracy of the measurement.

Calculations . As in most cases it is not possible to work under optimum reaction conditions, good laboratory practices recommend performing calibration test by using the calibrator indicated by the manufacturer of the reagent. Labtest indicates the calibrator Calibra H - Ref. 80 for calibrating the CHOLINESTERASE Liquiform system.

$$\Delta A/\text{minute Test or Calibrator} = (A_1 - A_2)/3$$

$$\text{Factor} = \frac{\text{Calibrator Activity}}{\Delta A/\text{min Calibrator}}$$

$$\text{CHOLINESTERASE (U/L)} = \Delta A/\text{min Test} \times \text{Factor}$$

Example

Test
$A_1 = 1.733$ $A_2 = 1.606$

$$\Delta A/\text{min Test} = \frac{1.733 - 1.606}{3} = 0.042$$

Calibrator
$A_1 = 1.716$ $A_2 = 1.552$

$$\Delta A/\text{min Calibrator} = \frac{1.716 - 1.552}{3} = 0.055$$

Calibrator Activity (U/L) = 4,100

$$\text{Factor} = \frac{4{,}100}{0.055} = 74{,}545$$

CHOLINESTERASE activity (U/L) = 0.042 x 74,545 = 3,131U/L

When optimal reaction conditions are met as mentioned above, you can choose to use the factor 74,400.

Calibration . Use the calibrator Calibra H - Ref 80, Labtest.

Calibrations range
When the internal quality control indicates.
When using fresh batch of reagents.
When using new reagent bottles of the same batch, if a new calibration has been performed during the use of the preceding bottle.

Operatinal range . The measurement result is linear between 80 and 20,000 U/L. For higher values, dilute the sample with 150 mmol/L NaCl (0.85%). Perform a new measurement and multiply the result by the dilution factor.

Internal quality control . The laboratory must maintain a program of internal quality control clearly defining the applicable regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and record of activities. Control materials should be used to monitor the inaccuracy in the measurement and calibration deviations. It is suggested that the specifications for the coefficient of variation and total error be based on the components of biological variation (BV)[3, 4, 5].

Reference range[6] . These values should be used only as a guide. It is recommended that each laboratory establish its own reference range in the population served.

Adults	Values (U/L)
Women	3930 - 10800
Men	4620 - 11500

Conversion: Conventional Units (U/L) x 16.7 = IS units (nkat/L).

Performance features[7]

Recovery studies . Samples with known cholinesterase activity were tested with the CHOLINESTERASE Liquiform system, obtaining the following results:

Activity (U/L)

Expected	Found	Recovery (%)
12763	12967	101.6
3483	3616	103.7
2396	2471	103.1
1583	1533	103.2

The average total systematic error obtained was equal to 2.83%. The total systematic error obtained was smaller than the total systematic error of the desirable specification in the components of the Biological Variation, which is ≤±4.8%.

Studies comparing methods . The proposed method was compared with another product of similar methodology, and the following results were obtained:

	Comparative Method	Cholinesterase Liquiform
Samples	40	40
Concentration range (U/L)	1335 - 10356	1344 - 10297
Regression equation	Labtest Method = 0.9978 x Comparative Method - 23.8	
Correlation coefficient	0.999	

By using the regression equation, the estimated systematic error (bias) is equal to 1.01% for a sample with cholinesterase activity equal to 3,000 U/L, 0.52% for a sample with cholinesterase activity equal to 8,000 U/L, and 0.39% for a sample with cholinesterase activity equal to 14,000 U/L. These errors are smaller than the analytical systematic error of the desirable specification based on the biological variation, which is ≤±4.8%.

Accuracy studies . The accuracy studies were conducted using samples with activity values equal to 2,881, 8,080 and 13,925 U/L.

Imprecision - Within Run

	N	Mean	SD (U/L)	(%) CV
Sample 1	20	2881	39.8	0.59
Sample 2	20	8080	27.4	0.35
Sample 3	20	13925	44.3	0.36

Imprecision - Run-to-Run

	N	Mean	SD (U/L)	(%) CV
Sample 1	20	2881	50.7	1.16
Sample 2	20	8080	82.7	0.92
Sample 3	20	13925	215.8	1.08

The total inaccuracy obtained for samples meets the specification desirable for total inaccuracy based on biological variation, which is ≤3.1%.

The total error (random error + systematic error) estimated in activities equal to 2,881, 8,080 and 13,925 U/L are equal to 2.92, 2.04 and 2.17%, respectively.
The results indicate that the method meets the specification desirable for the total error (≤±9.8%) based on desirable components of the Biological Variation.

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Methodology sensitivity . A sample containing no cholinesterase was used to calculate the detection limit of the assay, having found a value of 76.3 U/L equivalent to a mean of 10 tests plus three standard deviations.

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.

Reference

1. Dietz AA, Rubinstein HM, Lubrano T. Clin Chem 1973; 19(11):1309-1313.

2. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37 degrees C. II. Cholinesterase (acylcholine acylhydrolase, EC 3.1.1.8). Working Group of Enzymes, German Society for Clinical Chemistry. Eur J Clin Chem Clin Biochem 1992; 30(3):163-170.

3. Ricos C, Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation. Disponível em: http://westgard.com/biodatabase1.htm (acesso em 10/09/2012).

4. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.

5. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem 1981; 27:493-501.

6. Recommendation of the German Society for Clinical Chemistry. Standardization of methods for the estimation of enzyme activities in biological fluids: Standard Method for the determination of Cholinesterase activity. JClin Chem Clin Biochem 1992;30:163-70.

7. Labtest: Data on file.

8. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, 2.ed. Philadelphia: W.B. Saunders Company, 1994. p. 788-96.

Presentation

Product	Reference	Content	
Cholinesterase Liquiform	139-1/30	R 1	1 X 24 mL
		R 2	1 X 6 mL

Application procedures using Cholinesterase Liquiform are available for **various automated systems.**

The number of tests in automated systems **depends on the programmed parameters.**

Consumer information

[Warranty conditions]

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

CE

Labtest Diagnóstica S.A.

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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		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		

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