Cholinesterase B – Instructions for use (IFU)

Manufactured exclusively for: ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 4 x 50 mL - R2: 4 x 9 mL • REF A-R0200000400

R1: 3 x 13,9 mL – R2: 1 x 7 mL • REF R3330000015

IVD CE

INTENDED USE

Product for use in the quantitative determination in vitro of Cholinesterase activity in human serum and plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

The Cholinesterase enzyme is synthesized in the liver. It is present in the pancreas, myocardium and serum. Cholinesterase activity in the serum is the result of the activity of 13 isoenzymes. It is an index of liver function, although it is seen to be altered only in cases of very advanced hepatitis or cirrhosis. Other clinical situations in which an alteration in the cholinesterase concentration can be seen are poisoning by organic compounds (anticryptogamic substances) and some forms of anemia. The activity of this enzyme is also determined before subjecting patients to treatment with muscle-relaxants containing succinylcholine. In 1949 G.B. Koele (1) used acetylthiocholine in the histochemical localization of cholinesterase activity, making use of the enzyme's capacity to hydrolyse C-S bonds as well as C-O bonds. In 1961 G.L. Ellman et al. (2) developed a method for the determination of cholinesterase using as substrate acetylthiocholine and 5-5' dithio-bis-2-nitrobenzoic acid (DTNB) as revealer of the thiocholine which was freed enzymatically. Subsequently other authors (3,4) used butyrilthiocholine as substrate. The Cholinesterase B method is based on the optimization of the reagents proposed by Szasz, and they are presented in stabilized liquid form.

PRINCIPLE

Pseudocholinesterase (acylcholine acylhydrolase, EC 3.1.1.8) hydrolyzes butyrylthiocoline to butyric acid and thiocholine which in turn reacts with DTNB.
This reaction releases 5-thio-2-nitrobenzoic acid intensely colored yellow.

$$\text{Butyrilthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Butyrate} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{DNTB} \longrightarrow \text{5-thio-2 nitrobenzoate (yellow color)}$$

The rate of color appearance is directly proportional to cholinesterase activity.

REAGENTES

A-R0200000400- R1: 4 x 50 – R2: 4 x 9 mL
Reagent 1: n° 4 vials x 50.0 mL ready for use
Reagent 2: n° 4 vials x 9.0 mL ready for use
R3330000008 - R1: 3 x 13,9 – R2: 1 x 7 mL
Reagent 1: n° 3 vials x 13.9 mL ready for use
Reagent 2: n° 1 vials x 7.0 mL ready for use

Concentrations

Reagent 1:		
	Conc.	**U.M.**
Phosphate buffer pH 7.7± 0.2	50.5	mM
5,5'-dithio-bis-2-nitrobenzoic acid	0.25	mM
Reagent 2:		
Butyrildithiocholine iodide substrate	30.3	mM

Precautions

Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC directive 1272/2008 and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice

Reports of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Storage and stability

Store at 2 - 8°C and protect from direct light. When correctly stored, the reagents are stable up the expiry date reported on the label. A slight variation in the composition of the reagent may occur between batches, but this has no effect on the test results. After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature.

Reagent Preparation

The reagents are liquid, ready to use. The solutions must be limpid with no evident precipitate. Pay attention to avoid bacterial contamination during use. The stability of the reagents are 30 days if closed, stored at 2 - 8°C and protect from direct light.

SAMPLE COLLECTION

Type of sample and storage

Use serum or plasma containing heparin or EDTA. Pseudocholinesterase is stable in the serum for 1 week at 2 - 8°C and for at least 3 months at - 20°.

Precautions

All human samples must be handled and disposed of as potentially infectious materials.

PROCEDURE

Quality control

Human control sera with known levels of CHE-B are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and abnormal control sera are available as "Normal control serum" code R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:
Cleanliness of glassware.

- Wavelength setting.
- Expiration date of reagents.

Automation

Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications not explicitly approved by. cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration

For calibration use the kit "Multicalibrator" code R0300000006. For the use on I.S.E. srl instruments Calibration every 10 days is recommended.

Traceability:

The CHE-B is visible in the insert of the calibration serum package.

Reaction conditions

Wavelength (primary): 405 nm
Temperature: 37°C

Technique

- Bring the reagents to room temperature and operate away from direct light.
- Reaction volumes can be varied proportionally.

	U.M.	**Calibration serum**	**Sample**
Reagent A	µL	1000	1000
Calibration serum	µL	10	-
Sample	µL	-	10
	U.M.	**Calibration serum**	**Sample**
Reagent B	µL	170	170

Mix gently then incubate at reaction temperature for 20 sec.
The reaction volumes can be varied proportionately, remaining unchanged on calculation. After incubation, read absorbance at 405 nm. Repeat readings every 30 seconds or every 60 seconds. At least 3 repetitions of reading in the chosen times are recommended. Determine the mean between the Δ D.O./min.

Results

Manual method

Calculation of Cholinesterase B concentration:

$$\frac{\Delta \text{ O.D. Sample}}{\Delta \text{ O.D. Calibration Serum}} \times \text{Calibration serum conc. (U/L)} = \text{U/L CHE-B}$$

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L of CHE-B

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Explanation of the formula:

$$\frac{Vt \times 1000}{M.E.C. \times O.P. \times Vc} = K - factor * \times \Delta\ O.D./min. = U/L\ CHE - B$$

*K-factor = 8847
where:
U/L = activity in international units per litre
Δ D.O./min. = change in absorbance per minute
Vt = total reaction volume (µL)
1000 = conversion of concentration per litre
C.M.E. = coeffic. micromolar of extinction of 5,5-dithio-bis-2-nitrobenzoate
13.3 cm2/µmol at 405 nm
P.O. = optical path (1,0 cm)
Vc = sample volume in the final reaction mixture (µL)

Reagents included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Controls and calibrators.

NORMAL VALUES
Serum or Plasma:
- 4700 – 14100 IU/L

Each laboratory must establish its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity
Cholinesterase is linear up to a concentration of ≥15000 IU/L and in the presence of higher concentrations, repeat the test by diluting the serum 1:10 in physiological saline and multiplying the final result by 10.

Specificity
The colour which develops is proportional to the amount of cholinesterase isoenzymes present in the sample and able to hydrolyse butyrildithiocholine.

Accuracy-Recovery
Amounts of Cholinesterase added to a serum matrix containing a known amount of Cholinesterase gave an average recovery of 109%.

Interference
No interference was caused by Bilirubin < 55 mg/dL, haemolysis < 10 g/L, lipemia < 900 mg/dL.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	IU/L	5113	155.9	3.05%	20
High	IU/L	7669	277.6	3.62%	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	IU/L	5113	158.6	3.10%	20
High	IU/L	7669	445.3	5.81%	20

Sensitivity
At λ 405 nm, a concentration of about 56.72 IU/L of CHE-B, in the conditions established for this test.

Comparative method
CHE-B reagent was compared with the method described by Ellman (2).
The following results were obtained: Linear regression: Y = 0.9640x + 1495; Correlation coefficient r = 0.867; Sample tested No. = 71.

Disposal of reagents
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:
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Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

Reference

1. Hallbach J, Klinische Chemie für den Einstieg. 1st ed Stuttgart: Thieme;2001. p. 143-4.
2. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
3. Recommendations 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. J Clin Chem Clin Biochem 1992;30:163-70.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000

REVISION	DATE	CHANGE
Rev.C	01/2024	Edit header and “Reagents” paragraph.