Cholinesterase B – Method Enzymatic
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
CHEMILAB	REF B81180221	n° 2 vials x 34 mL (R.A) n° 1 vial x 12 mL (R.B)

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 succynilcholine.

PRINCIPLE OF THE METHOD
Method Enzymatic (Butyrilthiocholine). Pseudocholinesterase (acylcholine acylhydrolase, EC 3.1.1.8) hydrolyses butyrilthiocholine to butyric acid and thiocholine which in turn reacts with DTNB. This reaction frees intensely yellow-coloured 5-thio-2-nitrobenzoic acid.

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

$$\text{Thiocholine} + \text{DTNB} \longrightarrow 5-\text{thio}-2\text{nitrobenzoate (Yellow Colour)}$$

The rate at which the colour forms is directly proportional to the cholinesterase activity.

Storage and stability
= storage temperature 2 - 8°C
If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentrations

Reagent A:		
	Conc.	U.M.
Phosphate buffer pH 7.7± 0.2	50.5	mmol/L
5,5'-dithio-bis-2-nitrobenzoic acid	0.253	mmol/L
Reagent B:		
Butyrildithiocholine iodide substrate	30.3	mmol/L

Reagents included in the kit
The reagent is described above.

Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting 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.

PROCEDURE
Quality control
Control sera with a known titer for Cholinesterase are commercially available for quality control, accompanied by certificates of analysis. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5 ml code B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be contained within the acceptability range.

Calibration
For calibration use the "Calibrator serum Sclavo" code B35181702.

Traceability
The CHE-B concentration is reported in the package insert supplied with the "Calibrator Serum."

SAMPLE
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°.

PREPARATION OF THE REAGENT
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 reagents are stable for 30 days if closed and stored at 2 - 8°C avoiding direct light. A slight variation in the colour, from batch to batch, does not affect the test results.

ANALYTICAL TECHNIQUE
For automatic procedures, please refer to the user manual and application notes of the CHEMILAB analyzer. All applications not approved by Sclavo Diagnostics cannot be guaranteed in terms of performance and must therefore be evaluated by the user.

Calculation of results
The results are automatically calculated by the analyzer using the calibration line. The analyzer automatically picks up the appropriate amount of a primary standard as set in the application method. The calibration line is obtained by interpolating the values obtained with an appropriate calculation algorithm.

REFERENCE RANGE
Serum or plasma: 4700 – 14100 U/L
Each laboratory should calculate its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
Cholinesterase is linear up to a concentration of 15000 U/L. 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.

Recovery
Commercial control sera were analyzed with the kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the following table.

Level	Replicates	Mean (U/L)	DS	CV%	Recovery
Low	5	4927	36,88	95,9	95,9%
High	5	5782	83,47	99,0	99%

Interference

Interference	Limits
Lipid	**900 mg/dL**
Bilirubin	**55 mg/dL**
Hemolysis	**10 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability				
Range	**Replicates**	**Mean (U/L)**	**DS**	**CV%**
Low	40	6161	68.6	1.11
High	40	7621	54.8	0.72
Total precision (Within-lab precision)				
Range	**Replicates**	**Mean (U/L)**	**DS**	**CV%**
Low	40	6162	117	1.91
High	40	7621	200	2.64

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Limit of Sensitivity
The limit of sensitivity was measured by analyzing scalar dilutions of a concentrated serum. Under the conditions established for this test the lowest detectable concentration is 212 U/L cholinesterase B

Comparison between methods
The method for Cholinesterase B was compared with a similar commercially available method, analyzing 91 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	101
Slope	0,75
Correlation Coeff. (R)	0,99

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	

REFERENCES

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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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