Total Cholinesterase – Trinder Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182540	n° 10 vials x 10 mL n° 10 vials (freeze-dried)

INTENDED USE

Product for the quantitative in vitro determination 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

Acetylcholinesterase, also known as true cholinesterase, is one of the two enzymes capable of hydrolyzing acetylcholine. True cholinesterase is localized in the erythrocytes, in the lungs, the spleen, in nerve endings and the grey brain matter. The other cholinesterase is acylcholine acylhydrolase (SChE), an enzyme which is present in various organs such as the liver, pancreas, heart, white brain matter and serum. Cholinesterase has a role as a diagnostic aid as an indicator of possible poisoning by insecticides, in the recognition of atypical forms of the enzyme, or as a test of hepatic function. A 40% drop in enzyme activity in serum occurs before the warning of initial symptoms and an 80% drop before the onset of neuromuscular effects.

PRINCIPLE OF THE METHOD

Method Benzoylcholine.This procedure makes use of benzoylcholine as substrate. The reagent contains dibucaine as inhibitor for the differentiation of the "normal" and "atypical" forms of cholinesterase.

$$\text{Benzoylcholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Choline} + \text{Benzoic acid}$$

$$\text{Choline} + O_2 \xrightarrow{\text{CholineOxidase}} \text{Betaine} + 2\,H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Chinone}$$

The amount of chinone formed has a red colour, the intensity of which is read at a λ 510 nm and is proportional to the concentration of non-inhibited Cholinesterase in the test sample.

Storage and stability

= storage temperature 2-8°C

stored at 2-8 ° C avoiding direct light, the reagents are stable until the expiration date printed on the label.

Concentrations

Reagente pronto per l'uso		
	Conc.	**U.M.**
Phosphate buffer pH 7.9	100	mmol/L
Phenol	10.0	mmol/L
Benzoylcholine	1.00	mmol/L
4-aminophenazone (4-AAP)	0.40	mmol/L
Choline oxidase	1300	IU/L
Peroxidase	150	IU/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 of total CHE are commercially available for quality control, with values and confidence limits included. . Normal and pathological control sera Sclavo Diagnostics are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration

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

Traceability

The Total CHE concentration is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE COLLECTION

Type of sample and storage

Fresh non hemolysed serum samples should be used. Use serum or plasma with heparin or EDTA. Cholinesterase in the serum is stable for a week at 2-8°C and 12 months at – 20°C.

REAGENT PREPARATION

Pour the contents of the Reagent A Vial into the Vial of Freeze-Dried Reagent B. Mix gently by inversion until it is completely dissolved, then pour the solution back in the vial of Reagent A. Leave the solution to rest for 15 minutes before use. After reconstitution the reagents are stable for 5 days if closed and stored at 2-8°C. Slight variations in color from batch to batch, do not affect the test results.

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / C. Validated applications are available for Sclavo Konelab® - Indiko instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	510 nm
Temperature:	37°C
Reaction	End point (Increasing reaction)

Technical - Procedure with Serum as starter

- Bring the reagents to reaction temperature and operate away from direct light.

-	U.M.	Calibr.Serum	Campione
Reagent	µL	1000	1000
Calibr.Serum	µL	10	-
Sample	µL	-	10

Mix gently then incubate at reaction temperature for 180 sec.
After incubation read absorbance at 510 nm. Repeat readings every 30 seconds or every 60 seconds. At least 3 repetitions of reading in the chosen times are recommended. Determine the average between Δ D.O./min. **The reaction volumes can be varied proportionately, without altering the results.**

Calculation of the results obtained with a multiplication factor

Δ O.D./min x K-factor* = U/L of Total CHE
Explanation of the calculation:

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

*K-factor = 14659

where:
U/L = activity in serum in international units
Δ O.D./min. = variation in absorbance per minute
Vt = total reaction volume (µL)

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1000 = conversion to the concentration per liter
M.E.C. = micromolar extinction coefficient of chinone dye 6.89 cm²/ µmol λ 510 nm
O.P. = optic path (1cm)
Vc = sample volume in the mixture (µl)

Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration curve/line is obtained through a special validated algorithm.

REFERENCE RANGE
Human serum or plasma: 2260-7550 IU/L
Each laboratory must establish its own normal values on the basis of its local catchment area.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
The method is linear up to a concentration of 5377 IU/L at λ 510 nm, in the case of samples with concentrations higher than 5377 IU/L, repeat the test by diluting the serum in physiological saline and multiplying the final result for the dilution factor.

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 table below.

Level	Replicates	Mean(U/L)	DS	CV%	Recovery
Low	5	4633	114,19	2,5	95,9%
High	5	4112	255,61	6,2	99%

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	1521	100	6,57	30
High	U/L	3124	82,1	2,62	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	2113	58,2	2,75	20
High	U/L	3015	59,7	1,98	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is 37IU/L of inhibited cholinesterase in the conditions established for this test.

Comparison between methods
The method was compared with a similar commercially available method, analyzing 200 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-33.231
Correlation Coeff. (R)	0.982

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	

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

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