Inhibited Cholinesterase – Method Benzoylcholine

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182541	n° 10 vials x 10 mL n° 10 vials (freeze-dried)

INTENDED USE

Product for use in the quantitative determination in vitro of the "dibucaine number" of Cholinesterase, by means of the determination of the cholinesterase activity with and without inhibition of dibucaine in human serum or 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. Succynilcholine (suxanmethonium), a muscle-relaxing drug used in surgical anesthesia, is also hydrolyzed by cholinesterase and its pharmacological effect only persists for the time necessary to complete the surgical operation. In subjects with low levels of the enzyme or in those with weakly active variants, the drug is not eliminated from the organism with sufficient rapidity and the patient may enter a period of prolonged apnea requiring mechanical ventilation until the drug has been completely eliminated by other routes. The gene which controls the synthesis of SChE can exist in several allelic forms. Four of the most common forms are denominated E_1^u, E_1^a, E_1^f, e E_1^s. The most common normal phenotype is indicated by the code E_1^u .The E_1^a gene is indicated as an atypical gene; the serum of homozygotic subjects is only weakly active towards the majority of cholinesterase substrates. The E_1^f gene is particularly resistant to inhibition by fluoride. The E_1^s (silent) gene is associated with the absence of enzyme or the presence of a protein with minimum or no catalytic activity. In the presence of the local anesthetic dibucaine, the activity of SChE is inhibited to a greater extent compared to the atypical SChE variants. Patients can be assigned to one of the following three groups on the basis of the degree of inhibition:

Ist inhibition group >70%: these are subjects which are homozygotic for the normal SChE in both genes

II nd inhibition group 40/70%: heterozygotic subjects with a gene for normal SChE and one for atypical SChE

III rd inhibition group: subjects which are homozygotic for the atypical variant for both genes.

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.

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

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

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

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
Dibucaine	0.35	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.
90. 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 Inhibited CHE are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera 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 Inhibited CHE concentration is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE COLLECTION

Type of sample and storage

Fresh non haemolysed serum samples should be used. Use serum or plasma with heparin or EDTA. Cholinesterase in the serum is stable for a 5 week at 4 - 20°C and 1 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 composition 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)

Sclavo Diagnostics International
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • fax +39 0577 390 444
www.sclavodiagnostics.com

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Technical - Procedure with Serum as starter

Bring the reagents to room 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 alteration of results.**

Calculation of the results obtained with a multiplication factor

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

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

*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)
1000 = conversion to the concentration per liter
M.E.C. = micromolar extinction coefficient of chinone dye 6.89 cm^2/ µmol λ 510 nm
O.P. = optic path (1cm)
Vc = sample volume in the mixture (µl)

Calculation of the Dibucaine Number

Formula = result in % Dibucaine number

$$100 - \frac{\text{Inhibited CHE Activity}}{\text{Total CHE Activity}} \times 100 = \% \text{ Dibucaina Number}$$

Automation

The results are automatically calculated by the analyzer based on the calibration curve/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

% Dibucaine Number in human serum or plasma.

- Normal homozygotic subjects: > 70 %
- Heterozygotic subjects: 40 -70%
- Atypical homozygotic subjects: < 30 %

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 3500 IU/l at λ 510 nm, in the case of samples with concentrations higher than 3500 IU/l, repeat the test by diluting the serum in physiological saline and multiplying the final result for the dilution factor.

Recovery - Trueness

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.

Livello	Replicati	Media (U/L)	DS	CV%	Recovery
Basso	5	1174	33,77	2,9	95,9%
Alto	5	1075	39,55	3,7	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	688	7.40	1.07	30
High	U/L	325	3.70	1.13	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	691	1.90	0.27	20
High	U/L	345	2.70	0.78	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	

REFERENCES

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2. **Wroblewsky F., Ladue J.S.,** (1965). Proc. Soc. Exper. Biol and Med, 91:569
3. **NCCLS Document,** "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. **EU-Dir 1999/11** Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
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8. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
9. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07
10. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

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

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