Colinesterasa

AA

For the determination of cholinesterase activity in serum or plasma

SUMMARY

The existence of two cholinesterases has been proved: one is Acetylcholinesterase or "true Cholinesterase" (Acetylcholine hydrolase, E.C. 3.1.1.7), which is found in erythrocytes and in cholinergic nerve terminals. The other one is the butyrylcholine esterase or pseudocholinesterase (E.C. 3.1.1.8), which is found in plasma, liver, smooth muscle and fat cells.

Serum or plasma cholinesterase (ChE) or pseudocholinesterase, is associated to the following clinical conditions:

1) It is considered as an indicator of hepatic function, especially in chronic pathologies. A good correlation between the increase of GOT (AST) and the decrease of ChE in infectious hepatitis can be observed.

2) Its decrease indicates intoxication by organophosphate pesticides, which are ChE inhibitors.

3) In some individuals, sensitive to succinyl choline -a muscle relaxant administered during anesthesia- a prolonged post-anesthetic apnea is observed, sometimes with fatal outcomes. This coincides with the presence of a genetic variation of ChE ("atypical") unable to hydrolyze succinyl choline. In normal individuals this drug is hydrolyzed "in vivo" by ChE, in 1 to 4 minutes. Thus, prolonged apnea is also related to low levels of total ChE. There are methods of differential inhibition that allow the detection of carriers of this atypical cholinesterase.

PRINCIPLE

$$\text{butyrylthiocholine} + H_2O \xrightarrow{\text{ChE}} \text{thiocholine} + \text{butyrate}$$

$$\text{thiocholine} + \text{DTNB} \longrightarrow \text{2-nitro-5-mercaptobenzoate}$$

PROVIDED REAGENTS

A. Reagent A: vials containing 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in phosphate buffer for a final pH 7.7.
B. Reagent B: vials containing S-butyrylthiocholine iodide.
C. Reagent C: Reagent A diluent aqueous solution with proper preservatives.
D. Reagent D: Reagent B diluent aqueous solution with proper preservatives.

Final concentrations

S-butyrylthiocholine iodide..... 6 mmol/l
DTNB..... 0.25 mmol/l
phosphate buffer.....50 mmol/l; pH 7.7

NON-PROVIDED REAGENTS

Saline solution.

INSTRUCTIONS FOR USE

Reagent A: reconstitute with stated volume of Reagent C. Homogenize by inversion until complete dissolution and date.
Reagent B: reconstitute with stated volume of Reagent D. Homogenize by inversion until complete dissolution and date.
Reagent C: ready to use.
Reagent D: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10°C) until the expiration date printed on label.
Reconstituted Reagent A: stable for 6 weeks in refrigerator (2-10°C). Store protected from direct light exposure.
Reconstituted Reagent B: stable for 6 weeks in refrigerator (2-10°C). Store tightly capped.
Reagent C and D: once opened, should remain neither uncapped nor without refrigeration for long periods of time. Avoid contamination.

SAMPLE

Serum or plasma
a) Collection: obtain the sample in the usual way.
b) Additives: when using plasma, use heparin or EDTA-based anticoagulants (Wiener lab.'s Anticoagulante W).
c) Known interfering substances: no interferences are observed from hemoglobin up to 1000 mg/dl, triglycerides up to 25 g/l, bilirubin up to 200 mg/l and heparin up to 50 U/ml. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. Samples could be stored up to one week in refrigerator (2-10°C) without added preservatives.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Spectrophotometric square cuvettes.
- Kahn or hemolysis tubes.
- Water bath at selected reaction temperature.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 405 nm
- Reaction temperature: 25, 30 or 37ºC. The temperature of the reaction should be strictly maintained at the selected temperature. See the corresponding REFERENCE VALUES for each temperature.
- Reaction time: 3 minutes
- Sample volume: 10 ul
- Final reaction volume: 1.51 ml

Sample and reagent volumes may be proportionally changed, without altering calculation factors.

PROCEDURE

Preincubate the necessary volume of reconstituted Reagent B at the selected temperature for a few minutes. In a cuvette at the selected temperature, add:

Reconstituted Reagent A	1.2 ml
Pre-incubate for 2 minutes. Then add:	
Sample	10 ul
Homogenize and immediately add:	
Reconstituted Reagent B	0.3 ml

Mix, incubate for 15 seconds and read the absorbance with simultaneous stopwatch start. Reread after exactly 30 and 60 seconds. Determine absorbance's average difference every 30 seconds (ΔA/30 sec) subtracting each reading from the previous one and averaging values. Use this mean for calculations.

CALCULATIONS

Cholinesterase (U/l) = ΔA/30 sec x 22210

REFERENCE VALUES

25ºC	30ºC*	37ºC
Children, men and women over 40 years old:		
3500-8500 U/l	4300-10500 U/l	5500-13400 U/l
Women between 16-39 years old, not pregnant and not ingesting oral contraceptives:		
2800-7400 U/l	3450-9100 U/l	4400-11700 U/l
Women between 18-41 years old, pregnant or ingesting oral contraceptives:		
2400-6000 U/l	3000-7400 U/l	3800-9500 U/l

* Values calculated using the following temperature conversion factors:

25-30ºC: 1.23
25-37ºC: 1.58

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

Cholinesterase (kU/l) = Cholinesterase (U/l) x 0.001

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known cholinesterase activity.

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Reagents are particularly sensitive to contamination. Use only thoroughly clean and dry micropipettes for measurements.

PERFORMANCE

a) Precision: processing according to EP5A protocol of NCCLS (National Committee on Clinical Laboratory Standards), the following values were obtained:

Within-run precision

Level	S.D.	C.V.
8863 U/l	± 100.75 U/l	1.14 %
4811 U/l	± 46.69 U/l	0.97 %

Total-run precision

Level	S.D.	C.V.
8863 U/l	± 177.68 U/l	2.00 %
4811 U/l	± 94.79 U/l	1.97 %

b) Detection limit: depends on the photometer used and the wavelength. In spectrophotometer with 1 cm optical length square cuvettes, for a ΔA/30 sec of 0.001, the minimum detectable activity change will be of 22 U/l.
c) Dynamic range: if ΔA/30 sec is over 0.400, repeat assay with Sample diluted 1/2 with saline solution. Correct results accordingly.
d) Linearity: the reaction is linear up to 17000 U/l. For higher values, dilute the sample with saline solution, repeat the test and multiply the result by the dilution factor.

PARAMETERS FOR AUTOANALYZERS

For programming instructions of other analyzer, check the user manual of the analyzer in use.

WIENER LAB. PROVIDES

78 ml: 3 x → 20 ml Reagent A
3 x → 6 ml Reagent B
1 x 60 ml Reagent C
1 x 20 ml Reagent D
(Cat. Nº 1241403)

REFERENCES

- Szasz, G. - Clin. Chim. Acta 19:191 (1968).
- Knedel, M. y Böttger, R. - Klin. Wochr. 45/325 (1967).
- Dietz, A.S. et al. - Clin. Chem. 19/11:1309 (1973).
- den Blaauwen, D.H. et al. - J. Clin. Chem. Biochem. 21/6:381 (1983).
- Ellman, G. - Archives of Biochemistry and Biophysics 82:70 (1959).

- Newman, M.A., Que Hee S.S. - Clin. Chem. 30/2:308 (1984).
- Tietz Textbook of Clinical Chemistry - Burtis, C.; Ashwood, E. (3º Edition) WB Saunders, 1999.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 3rd ed., 1990.
- NCCLS (National Committee for Clinical Chemistry Standards) - Document "Evaluation of the Linearity of Quantitative Analytical Methods", EP6-P (1986).
- NCCLS (National Committee for Clinical Chemistry Standards) - Document "Evaluation of Precision Performance of Clinical Laboratory Devices", EP5-A (1999).

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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