



# Cholinesterase

For cholinesterase determination in serum or plasma

## SUMMARY

The existence of two cholinesterases has been proved: one is Acetylcholinesterase called true cholinesterase (Acetylcholine acetylhydrolase, E.C. 3.1.1.7), which is found in erythrocytes and in cholinergic nerve endings. The other one is the butyrylcholine esterase or pseudo-cholinesterase (E.C. 3.1.1.8), which is found in plasma, liver, smooth muscle and fatty cells. Serum or plasma cholinesterase (ChE) or pseudo-cholinesterase, is associated to the following clinical conditions:

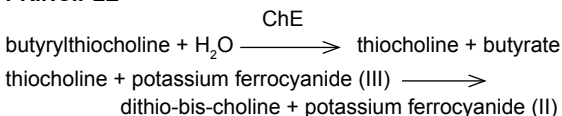
1) It is considered as an indicator of hepatic function, especially in chronic pathologies. A good correlation between the increase of GOT (AST) and the decrease of ChE in infectious hepatitis can be observed.

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



## PROVIDED REAGENTS

**A. Reagent A:** 73 mM pyrophosphate buffer, 2.4 mM potassium ferrocyanide (III), pH 7.7.

**B. Reagent B:** 10 mM Goods buffer solution, 92 mM butyrylthiocholine, pH 4.0.

## NON-PROVIDED REAGENTS

- Wiener lab's **Calibrador A plus**
- Saline solution (9 g/l NaCl)

## INSTRUCTIONS FOR USE

**Provided Reagents:** ready to use.

## WARNING

Reagents are for diagnostic "in vitro" use.

Use the reagents according to the working procedures for clinical laboratories.

All reagents and samples should be discarded according to current regulations.

## STABILITY AND STORAGE INSTRUCTIONS

**Provided Reagents:** stable at 2-10°C until the expiration date stated on the box. Once opened, they should not remain uncapped or outside the refrigerator for extended periods of time. Avoid contamination. Protect from light.

## SAMPLE

Serum or heparinized plasma or plasma with EDTA

**a) Collection:** obtain in the usual way.

**b) Additives:** when using plasma, heparin or EDTA (Wiener lab's **Anticoagulante W**) are recommended as anticoagulant for its collection.

**c) Known interfering substances:** no interferences have been observed with bilirubin up to 500 mg/l, triglycerides up to 14 g/l and hemoglobin up to 1000 mg/l. Refer to Young, D.S. in references for drugs' effect on the present method.

**d) Stability and storage instructions:** freshly collected samples are preferable. Samples could be stored for up to one week at 2-10°C or for up to 1 year at -20°C without adding preservatives.

## REQUIRED MATERIAL (non-provided)

- Volumetric material for measuring stated volumes
- Automated analyzer

## PROCEDURE

(Automated analyzer)

Below is a general procedure for Cholinesterase in automated analyzers. For programming instructions check the user's manual of the automated analyzer in use.

<b>Sample or Calibrator</b>	4 ul
-----------------------------	------

<b>Reagent A</b>	150 ul
------------------	--------

Incubate during 300 seconds at 37°C

<b>Reagent B</b>	30 ul
------------------	-------

Incubate during 120 seconds at 37°C. Measure initial absorbance at 405 nm ( $A_1$ ). After exactly 90 seconds measured with stopwatch, register a second measurement ( $A_2$ ). To obtain cholinesterase result in U/l, multiply absorbance difference ( $\Delta A = A_2 - A_1$ ) by factor.

## CALIBRATION

Calibrador A plus is processed in the same way than the samples, the corresponding factor is calculated based on it. Enter the calibrator concentration value each time the lot is changed.

## QUALITY CONTROL METHOD

For each test, process 2 levels of quality control material (**Standatrol S-E 2 niveles**) with known activities of cholinesterase.

## REFERENCE VALUES

### Serum or plasma

Children, men and women > 40 years old: 5320 - 12920 U/l  
Women between 16 and 39 years old, not pregnant and not taking oral contraceptives: 4260 - 11250 U/l

Women between 18 and 41 years old, pregnant or taking oral contraceptives: 3650 - 9120 U/l

It is recommended that each laboratory establish its own reference values, taking into account sex, age, eating habits, medications and other population factors.

## SI SYSTEM UNITS CONVERSION

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

## PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

To preserve reagents' integrity avoid all forms of contamination, only using thoroughly clean and dry micropipettes for measurement. It is recommended to use Wiener lab's **Standatrol S-E 2 niveles** as quality control material. The use of controls from other manufacturers may yield different values for certain ranges because they depend on the method or system used

## PERFORMANCE

a) **Precision:** based on EP15A protocol from CLSI, the following coefficients of variation were obtained as estimators of the intra-assay (CV<sub>i</sub>) and total (CV<sub>t</sub>) precision:

Level	CV <sub>i</sub>	CV <sub>t</sub>
2553.3 U/l	1.4%	1.5%
4282.7 U/l	2.8%	2.5%
6790.7 U/l	1.5%	1.5%

b) **Detection limit:** 70 U/l

c) **Quantification limit:** 262 U/l

d) **Linearity:** reaction is linear up to 14000 U/l. For higher values, dilute the sample with saline solution (9 g/l NaCl), repeat the assay and multiply the result by the dilution factor.

## PARAMETERS FOR AUTOMATIC ANALYZERS

For programming instructions, refer to the User Manual of the automated analyzer in use. For calibration use **Calibrador A plus** separately provided by Wiener lab.

## WIENER LAB PROVIDES

60 ml: - 1 x 50 ml Reagent A  
- 1 x 10 ml Reagent B  
(Cat. N° 1999704)

60 ml: - 1 x 50 ml Reagent A  
- 1 x 10 ml Reagent B  
(Cat. N° 1009278)

60 ml: - 1 x 50 ml Reagent A  
- 1 x 10 ml Reagent B  
(Cat. N° 1009350)

120 ml: - 2 x 50 ml Reagent A  
- 1 x 20 ml Reagent B  
(Cat. N° 1009607)

## REFERENCES

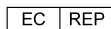
- Young D. Effects of Preanalytical Variables on Clinical Laboratory Test, editado por AACC, second edition, 1997.
- Young D. and Friedman R. Effects of Disease on Clinical Laboratory Test, editado por AACC, third edition, 1997.
- Young D. Effects of Drugs on Clinical Laboratory Test, AACC, editado por AACC, fourth edition, 2001.
- Burtis - Ashwood. Tietz Fundamentals of Clinical Chemistry, editado por W.B. Saunders Co., fifth edition, United States of America ,2001.
- Bergmeyer H. U. Methods of Enzymatic Analysis, Vol V 3<sup>rd</sup>.Edition.
- User Verification of Performance for Precision and Trueness; Approved Guideline - Second Edition. CLSI EP15-A2 Vol. 24 N° 25, 2004.
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI EP6-A Vol. 23 N° 16, 2003.
- Method Comparison and Bias estimation Using Patient Samples; Approved Guideline - Second Edition. CLSI EP9-A2 Vol. 22 N° 19, 2002.
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI EP17-A Vol. 24 N° 34, 2004.
- den Blaauwen DH, Poppe WA, Tritschler W. - J Clin Chem Clin Biochem 21:381-386, 1983.

# Symbols

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



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



Authorized representative in the European Community



"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution



Contents



Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use



Calibrator



Control




Positive Control



Negative Control



Catalog number

 Wiener Laboratorios S.A.I.C.  
Riobamba 2944  
2000 - Rosario - Argentina  
<http://www.wiener-lab.com.ar>  
Dir. Téc.: Viviana E. Cétola  
Biochemist  
A.N.M.A.T. Registered product  
PM-1102-50



**Wiener lab.**

2000 Rosario - Argentina