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

\[
\text{ChE} \quad \text{butyrylthiocholine} + \text{H}_2\text{O} \xrightarrow[]{} \text{thiocholine} + \text{butyrate} \\
\text{thiocholine} + \text{potassium ferrocyanide (III)} \xrightarrow[]{} \text{dithio-bis-choline} + \text{potassium ferrocyanide (II)}
\]

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.

<table>
<thead>
<tr>
<th>Sample or Calibrator</th>
<th>4 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>150 ul</td>
</tr>
<tr>
<td>Incubate during 300 seconds at 37°C</td>
<td></td>
</tr>
<tr>
<td>Reagent B</td>
<td>30 ul</td>
</tr>
<tr>
<td>Incubate during 120 seconds at 37°C. Measure initial absorbance at 405 nm (A), After exactly 90 seconds measured with stopwatch, register a second measurement (A'). To obtain cholinesterase result in U/l, multiply absorbance difference (A = A' - A) by factor.</td>
<td></td>
</tr>
</tbody>
</table>
CALIBRATION
Calibrator 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 (CVi) and total (CVi) precision:

<table>
<thead>
<tr>
<th>Level</th>
<th>CVi</th>
<th>CVt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2553.3 U/l</td>
<td>1.4%</td>
<td>1.5%</td>
</tr>
<tr>
<td>4282.7 U/l</td>
<td>2.8%</td>
<td>2.5%</td>
</tr>
<tr>
<td>6790.7 U/l</td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

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. Nº 1009278)
60 ml: - 1 x 50 ml Reagent A
 - 1 x 10 ml Reagent B
(Cat. Nº 1009350)
120 ml: - 2 x 50 ml Reagent A
 - 1 x 20 ml Reagent B
(Cat. Nº 1009607)

REFERENCES
- Young D. Effects of Preanalytical Variables on Clinical Laboratory Test, editado por AACC, second edition, 1997.
Symbols

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

- **Σ**
  - Contains sufficient for <n> tests

- **Use by**

- **Temperature limitation (store at)**

- **Do not freeze**

- **Biological risks**

- **Volume after reconstitution**

- **Contents**

- **Batch code**

- **Calibr.**
  - Calibrator

- **CONTROL**
  - Control

- **CONTROL +**
  - Positive Control

- **CONTROL -**
  - Negative Control

- **REF**
  - Catalog number

- **Manufactured by:**

- **Harmful**

- **Corrosive / Caustic**

- **Irritant**

- **Consult instructions for use**