Cholinesterase Liquid

Kinetic colorimetric determination of cholinesterase according to the DGKC recommendations in serum and plasma

**REAGENT 1**: 2 x 50 mL  -  **REAGENT 2**: 1 x 20 mL

**REF 17019A**

**REAGENT 1**: 6 x 90 mL  -  **REAGENT 2**: 6 x 20 mL

**REF 17606**

**IVD**: in vitro diagnostic medical device  
**STANDARD / CALIBRATOR**: the term refers to the standard / the calibrator  
**REAGENT**: the term refers to the single reagent  
**CONTROL**: the term refers to the control

**PRECAUTIONS IN USE**

- In addition to the possible risk indications regarding the reactive components, reagents may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 88/379/EEC directives and following modifications and amendments about classification, labelling and packaging of dangerous preparations (reagents). However, it is recommended to handle reagents carefully, to avoid ingestion and contact with eyes, skin and mucous membranes and to use laboratory reagents according to good laboratory practice.

**SUMMARY**

There are two cholinesterases (CHE and ACHE) differing in substrate specificity, tissue of origin and biological role. The term cholinesterase (ACHE), also known as acetylcholine Acetylhydrolase EC 3.1.1.7, is found in erythrocytes, in lung and spleen and in grey matter of brain. The Pseudocholinesterase (CHE), also referred to as acylcholine Acylhydrolase EC 3.1.1.8, is found in serum, liver, pancreas, heart and white matter of brain. The assay of serum cholinesterase (CHE) is useful to diagnose: liver disorders, hepatitis, cirrhosis, carcinoma with metastasis, sensitivity to succinylcholine administration and pesticide poisoning. Levels decrease in all above cases.

**PRINCIPLE**

The method uses butyrylthiocholine as the specific substrate for cholinesterase (CHE). Cholinesterase catalyses the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine. Thiocholine reduces hexacyanoferrate (III) to hexacyanoferrate (II). The decrease in absorbance is directly proportional to CHE activity in the sample.

**REAGENTS**

Reagents, stored at 2-8 °C in unopened vials, are stable up to expiry date indicated on the package.

**REAGENTS must be limpid; do not use if turbid.**

Components of the kit and initial concentration of reactive components:

- **REAGENT 1**
  - pyrophosphate buffer 92 mmol/L pH 7.6, hexacyanoferrate (III) 2.5 mmol/L
- **REAGENT 2**
  - butyrylthiocholine 91 mmol/L

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

**CALIBRATION**

For the calibration, it is recommended to use following materials:

- Clin Chem Cal
  - Lyophilized calibration serum. For use, follow the instructions contained in the kit.  
  **REF 16550**  
  **4x3mL**

**QUALITY CONTROL**

The use of following control materials at different levels of analyte is recommended to verify test accuracy:

- Clin Chem Control 1
  - Lyophilized control serum. For use, follow the instructions contained in the kit.  
  **REF 16150**  
  **6x5mL**
- Clin Chem Control 2
  - Lyophilized control serum. For use, follow the instructions contained in the kit.  
  **REF 16250**  
  **6x5mL**

**SAMPLE**

Fresh serum, plasma (EDTA, heparin) not haemolyzed and promptly separated from the red blood cells. Do not use sodium fluoride as an anticoagulant because it inhibits cholinesterase. Collect samples in accordance with the NCCLS procedure reported in bibliography (1). Stability of the sample: 15 days at 2-8 °C.

**Instrumentation and materials required but not provided**

- Usual laboratory equipment
- Filters photometer or spectrophotometer

**ANALYTICAL PROCEDURE**

- Wavelength: 405 nm
- Pathlength: 1 cm
- Temperature: 37 °C
- Sample/REAGENT 1/REAGENT 2: 1/50/10

Reaction: kinetic (decrease)

Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.
Put into cuvette

<table>
<thead>
<tr>
<th></th>
<th>Reagent Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>20 µL</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>20 µL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes. Add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>REAGENT 2</td>
<td>0.2 mL</td>
</tr>
</tbody>
</table>

Mix carefully. Read the absorbance of Reagent Blank and Sample after 90 seconds and repeat the readings after exactly 30, 60 and 90 seconds. Calculate the mean of ΔA/30”.

**CALCULATION**

Subtract the mean ΔA/30” Reagent Blank from ΔA/30” sample and multiply the obtained ΔA/30” by the factor F valid for the above mentioned test conditions (wavelength, sample volume, reaction volume and pathlength).

CHE activity (U/L) = (ΔA/30” mean Sample - ΔA/30” mean Reagent Blank) x F*

F* = 131.6 x 10^3 at 405 nm

**REFERENCE VALUES**

Adults (37 °C)
- Male: 5100 - 11700 U/L
- Female: 4000 - 12600 U/L

In infants up to 6 months of age, cholinesterase activity is 40% to 50% higher than in adults. In young women, the enzyme activity is approximately 64% to 74% of that in adults males. The activity decreases during pregnancy. It is recommended that each laboratory establish its own expected range.

**PERFORMANCES**

Interferences: A number of substances have been reported to cause physiological changes in serum cholinesterase activity. Less than 5% of interference is observed for haemoglobin, bilirubin and triglycerides up to 500 mg/dL, 20 mg/dL and 1000 mg/dL, respectively.

Measuring range: 160 - 25000 U/L. If ΔA/30” is higher than 0.160 (CHE activity higher than 25000 U/L), samples must be diluted 1:10 with normal saline and result multiplied by 10.

Intra-Assay Precision: it was determined on Hitachi 717 Instrument on 20 replicates of each control (3 levels of cholinesterase tested - L1/L2/L3). Results were as follows:

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean U/L</th>
<th>SD</th>
<th>CV%</th>
<th>Mean Run to Run SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>3850</td>
<td>42.07</td>
<td>1.09</td>
<td>46.70</td>
<td>1.21</td>
</tr>
<tr>
<td>L2</td>
<td>6744</td>
<td>103.79</td>
<td>1.54</td>
<td>92.66</td>
<td>1.37</td>
</tr>
<tr>
<td>L3</td>
<td>13758</td>
<td>129.55</td>
<td>0.94</td>
<td>126.87</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Inter-Assay Precision: it was determined according to the NCCLS Document EP5-P protocol (3 different levels - L1/L2/L3). Results were as follows:

<table>
<thead>
<tr>
<th>Level</th>
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Sensitivity: 160 U/L. Sensitivity was calculated on 10 replicates of normal saline and reported as the “mean zero value + 3 SD”.

Accuracy: this cholinesterase test (y) was compared with a method using the same substrate (x). Results were as follows:

\[ N = 89 \quad r = 0.99412, \quad y = 0.86390 \times x + 204.2644 \]

**WASTE MANAGEMENT**

Reagents must be disposed off in accordance with local regulations.

**BIBLIOGRAPHY**