Quantitative determination of cholinesterase by means of photometric method.

Only for in vitro use in clinical laboratory (IVD).

Clinical Significance
Cholinesterases (CHE) are a group of enzymes preferably splitting choline and thiocholine esters. The names serum Cholinesterase and Pseudocholinesterase are also commonly used. The CHE measured in serum and plasma is synthesized in the liver and is determined in diagnosis of liver diseases, nephrotic syndrome and intestinal diseases with loss of protein (exudative enteropathy). Strongly decreased values can indicate intoxication by pesticides. Measurement of CHE is also a part of Pre-operative diagnostics as CHE is needed for the inactivation of muscle relaxants often used in surgeries.

Principle
Cholinesterase hydrolyses butyrylthiocholine under release of butyric acid and thiocholine. Thiocholine reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate (II). The decrease of absorbance is measured at 405 nm.

\[
\text{Butyrylthiocholine} + \text{H}_2\text{O} \rightarrow \text{Thiocholine} + \text{Butyrate}
\]

\[
2\text{Thiocholine} + 2[\text{Fe(CN)}_6]^{3-} + \text{H}_2\text{O} \rightarrow \text{Choline} + 2[\text{Fe(CN)}_6]^{4-} + \text{H}_2\text{O}
\]

Reagents

<table>
<thead>
<tr>
<th>R1</th>
<th>Pyrophosphate pH 7.6</th>
<th>95 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>Butyrylthiocholine</td>
<td>75 mmol/L</td>
</tr>
</tbody>
</table>

Storage Instructions and Reagent Stability
The reagent is stable up to the indicated month of expiry, if stored at 2–8 °C, protected from light and contamination is avoided. Do not freeze the reagents.

Reagent Preparation & Stability
Reagent is ready for use.

Materials required
- Spectrophotometer or colorimeter measuring at 340 nm,
- Thermostatic bath at 25°C, 30°C, 37°C (+0.1°C)
- Matched Cuvettes 1.0 cm light path,
- NaCl solution 9 g/L,
- General laboratory equipment.

Sample Collection and Preparation
Serum, heparin and EDTA plasma

<table>
<thead>
<tr>
<th>stability</th>
<th>: 2 weeks at 2-8°C</th>
<th>1 week at 15-25°C</th>
<th>6 months at -20°C</th>
</tr>
</thead>
</table>

Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

1. Assay Conditions:
   - Wave length: 405 nm
   - Cuvette length: 1 cm
   - Temperature: 37°C

2. Adjust the instrument to zero with distilled water or air.

3. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>Sample</th>
<th>20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>1000 µL</td>
</tr>
<tr>
<td>R2</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

4. Mix and incubate for 120 seconds.
5. Read initial absorbance (A) of the sample, start the stop watch and read absorbance at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance difference per minute (ΔA/min).

Calculation
\[ \Delta \text{Abs of sample} \times \text{Factor} = \text{Cholinesterase [U/L]} \]

*Factor = 68500

Quality Control
Commercially available normal and pathological control sera are recommended to monitor the performance of the procedure. If the controls are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal Quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

Reference Range

<table>
<thead>
<tr>
<th>Women</th>
<th>3930 - 10800 U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>4620 - 11500 U/L</td>
</tr>
</tbody>
</table>

It is suggested that each laboratory establish its own reference range.

Performance Characteristics

Measuring range: The test has been developed to determine CHE activities up to 20000 U/L
If such value is exceeded, the sample should be diluted 1+5 with NaCl solution (9 g/L) and results multiplied by 6.

Linearity: Upto 20000 U/L

Sensitivity: The lower limit of detection is 50 U/L.

Interferences
No interference was observed by ascorbic acid up to 30 mg/dl, bilirubin up to 45 mg/dl, hemoglobin up to 1000 mg/dL and lipemia up to 1400 mg/dL triglycerides.

Test Parameters

<table>
<thead>
<tr>
<th>Mode</th>
<th>Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>405</td>
</tr>
<tr>
<td>Sample Volume (µl)</td>
<td>20</td>
</tr>
<tr>
<td>Reagent Volume (µl)</td>
<td>1250</td>
</tr>
<tr>
<td>Delay Time (sec)</td>
<td>120</td>
</tr>
<tr>
<td>Read Time (sec)</td>
<td>180</td>
</tr>
<tr>
<td>Reaction Temperature (°C)</td>
<td>37°C</td>
</tr>
<tr>
<td>Reaction Direction</td>
<td>Decreasing</td>
</tr>
<tr>
<td>Factor</td>
<td>68500</td>
</tr>
<tr>
<td>Normal Low</td>
<td>3930</td>
</tr>
<tr>
<td>Normal High</td>
<td>10800</td>
</tr>
<tr>
<td>Linearity Limit</td>
<td>20000</td>
</tr>
<tr>
<td>Blank with Water</td>
<td>U/L</td>
</tr>
</tbody>
</table>

Manufactured by: Pathozyme Diagnostics, India
Marketed by

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