INTENDED USE
Quantitative determination of Dibucaine Number.

PRINCIPLE
Cholinesterase catalyzes the hydrolysis of butyrylthiocholine, forming butyrate and thiocholine, which reduces the ferricyanide ions to ferrocyanide. The decrease in absorbance is followed at 405 nm and it is proportional to cholinesterase activity in examined sample. Cholinesterase activity is measured with and without presence of dibucaine as inhibitor. Dibucaine number is calculated on the basis of inhibition percentage.

SAMPLE
Serum, plasma with heparin. The activity in the sample is stable 15 days at 2-8°C.

KIT COMPONENTS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Dibucaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>10 ml</td>
<td>16 mmol/l</td>
</tr>
<tr>
<td>(B)</td>
<td>1 ml</td>
<td>1,5 ml</td>
</tr>
</tbody>
</table>

The reagent is stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not use after expiry date. Once opened reagent is stable for 2 months at 2-8°C if contamination is avoided. Do not freeze.

Keep bottles closed when not in use.

REAGENT PREPARATION
Liquid Reagent, bring to room temperature (15-25°C) before use.

The Reagent Dibucaine is used with the Kit CHOLINESTERASE SL (REF. 0016-4153).

Reagent Preparation with inhibitor:

<table>
<thead>
<tr>
<th>REAGENT (A)</th>
<th>REAGENT (B)</th>
<th>DIBUCAINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ml CHOLINESTERASE SL</td>
<td>1 ml CHOLINESTERASE SL</td>
<td>1,5 ml</td>
</tr>
</tbody>
</table>

PRECAUTIONS AND WARNINGS
Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow. Use the normal precautions required in the laboratory. Dispose of waste according to local laws.

PROCEDURE
Wavelength: 405 nm
Lightpath: 1 cm
Temperature: 37°C
Reading: against distilled water
Method: Decreasing kinetic

pipette:

<table>
<thead>
<tr>
<th>REACTION WITH INHIBITOR</th>
<th>REACTION WITHOUT INHIBITOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (A+B) with Dibucaine</td>
<td>600 µl</td>
</tr>
<tr>
<td>Reagent(A+B) without Dibucaine</td>
<td>600 µl</td>
</tr>
</tbody>
</table>

Mix, incubate at 37°C for 1 minute, read the initial absorbance against water. Perform 3 readings at 30 seconds intervals. Calculate the average value of the absorbance variations per minute. (∆A/min).

Reaction volumes can be proportionally varied.

RESULTS CALCULATION
Perform calculation in Units per litre, multiplying the ∆A/min by the factor as it is indicated:

\[
\text{CHE total activity U/L: } \Delta A / \text{min} \times 65800 (\ast)
\]

\[
\text{CHE inhibited activity U/L: } \Delta A / \text{min} \times 65800 (\ast)
\]

(\ast) If you not use the Kit CHOLINESTERASE SL of Giesse Diagnostics (REF. 0016-4153), multiply by factor provided by the cholinesterase in use.

Dibucaine Number Calculation (DN):

\[
\text{DN} = 100 - \left( \frac{\text{CHE inhibited activity}}{\text{CHE without inhibitor activity}} \right) \times 100
\]

EXPECTED VALUES (DIBUCAINE)

Normal Subjects: 70 – 90 %
Heterozygous: 30 – 70 %
Homozygotes: 0 – 20 %

EXPECTED VALUES (CHOLINESTERASE)

Men: 3100 - 11200 U/L
Women: 4200 - 10800 U/L

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL
You must perform the controls at each kit’s use and verify that the values obtained are within the reference range reported in the operating instructions. For this purpose we recommend the use of control sera: PRECISENORM (REF.6000) and PRECISEPATH (REF.6001).

PERFORMANCE
Sensitivity: the sensitivity of the method is: 160 U/L
Linearity: the method is linear up to 25 KU/L. For higher values, dilute with saline 1:3 the sample and multiply the result by 3.

Precision intra-assay:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/L)</td>
<td>3850</td>
<td>6735</td>
</tr>
<tr>
<td>D5</td>
<td>57.04</td>
<td>155.22</td>
</tr>
<tr>
<td>CV %</td>
<td>1.46</td>
<td>2.30</td>
</tr>
</tbody>
</table>

Precision inter-assay:

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/L)</td>
<td>3850</td>
<td>6744</td>
</tr>
<tr>
<td>D5</td>
<td>46.70</td>
<td>92.66</td>
</tr>
<tr>
<td>CV %</td>
<td>1.21</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Interferences: bilirubin does not interfere up to 20 mg/dl. A moderate hemolysis does not interfere in the results.

Correlation against a reference method:

\[
y = 0.3194x + 527 \quad r = 0.9831
\]

REFERENCES