CH 50

Colorimetric assay for the quantitative determination of complement functionality in human serum

Only for in-vitro diagnostic use

GenWay Biotech, Inc.
6777 Nancy Ridge Drive
San Diego, CA  92121
Phone:  858.458.0866
Fax:  858.458.0833
http://www.genwaybio.com

Product Number: 40-521-475033 (96 Determinations)
1. INTRODUCTION

The primary utility of the CH50 in the practice of an allergist-immunologist is to screen for complement-deficiency associated immunodeficiency (primarily classic or terminal complement component deficiencies). Absent or significantly reduced individual complement components may result in infections, Neisseria meningitidis, or sepsis. A reduced CH50 in this situation warrants quantification and functional assays of individual complement components. Reduction of the CH50 occurs when individual complement component(s) are deficient or consumed.

2. INTENDED USE

Colorimetric method for quantitative determination of complement functionality in human serum.

3. PRINCIPLE OF THE ASSAY

The complex of β-galactosidase/anti-β-galactosidase is solubilised by serum through the deposition of C3b molecules. The formation of C3b is mediated by the alternative pathway, but it is accelerated by the activity of C3-convertase in the classic way. The quantity of dissociated β-galactosidase/anti-β-galactosidase complex is detectable by the enzymatic activity of the released β-galactosidase in the supernatant. By the use of o-nitrophenylgalactopyranoside (o-NPG) as substrate of β-galactosidase it is possible to detect the yellow reaction product o-nitrophenol at 420 nm (or 405 nm). The capacity of the serum sample to form C3b can therefore be expressed as enzyme activity of β-galactosidase.

4. MATERIALS

4.1. Reagents supplied

- Reference calibrator: 1 bottle containing 0.6 ml synthetic reference calibrator. The exact CH 50 value is reported on the label.
- Incubation buffer: 1 bottle containing 12 ml phosphate buffer (50mM, pH 7.35)
- Immunocomplex: 1 bottle containing 6 ml of β-galactosidase/anti-β-galactosidase complex
- ONPG Substrate Solution: 1 bottle containing 2.3 mM lyophilized o-NPG in phosphate buffer (15 mM, pH 7.0) (avoid any skin contact).
- Ethanediol: 1 bottle containing 1 ml Ethanediol
- Stop Solution: 1 bottle containing 7 ml sodium carbonate (16%) (avoid any skin contact).
- Microplate
- Low control: 1 bottle containing 0.6 ml of a synthetic control with a low solubilisation capacity. The exact value is reported on the label.
- High control: 1 bottle containing 0.6 ml of a synthetic control with a high solubilisation capacity. The exact value is reported on the label.

4.2. Materials and Equipment needed

- 37 °C incubator
- ELISA microwell plate reader, equipped for the measurement of absorbance at 420 or 405 nm
- Centrifuge for Eppendorf tubes(10000 - 13500 x g)
- Vortex
- Rolling mixer
- Micropipette, adjustable multichannel pipette, Sequential micropipette
- Distilled water
- Eppendorf tubes
- Timer
- Reference serum: 1 vial lyophilized DNOV096RS

5. STORAGE

Store all reagents at 2...8 °C in the dark.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (22...28°C) before starting the test run!
6.1. Immunocomplex
Use the reagent without any dilution. Before use mix well with vortex. Stable for 3 months at 2…8 °C.

6.2. ONPG Substrate
Add 10 ml of distilled water to the reagent. Once the reagent is dissolved, add 0.5 ml of Ethanediol. Stable for 2 months at 2…8 °C.

Note: For a better repeatability (inter-assay) we suggest to bring the substrate at room temperature (22…28°C) before use (avoid the dispersion of reagent just removed from the fridge)

7. SPECIMEN
The CH 50 assay can be performed in human serum only. Human serum is stable for one month if stored at -20°C (six months if stored at – 80°C).

8. ASSAY PROCEDURE
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.

Step 1 in Eppendorf tube
1. Dispense each serum sample, reference calibrator and a not solubilising control (no serum or reference serum is added) in Eppendorf tubes according to the following scheme:

<table>
<thead>
<tr>
<th></th>
<th>Reference serum (control)</th>
<th>Sample</th>
<th>Not solubilising control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation buffer</td>
<td>100 µl</td>
<td>100 µl</td>
<td>150 µl</td>
</tr>
<tr>
<td>Reference calibrator (control)</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample (serum)</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>Immunocomplex</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

2. Mix well and incubate 2 hours at 37°C.
3. Centrifuge at 10.000 – 13.500 x g for 15 min.
4. Transfer 50 µl supernatant of each tube into a well of the microplate. Be careful and avoid touching the pellet with the pipette!

Note: - avoid the suspension of the pellet
- do not shake the centifugate
- Take the supernatant slowly in order to avoid turbulences that cause suspension of the pellet

The pellet is composed of not solubilised immunocomplex with high enzymatic activity (β-galactosidase); the presence of a small quantity of pellet in the supernatant can cause false positive and erroneous values for the controls.

Step 2 in microplate

<table>
<thead>
<tr>
<th></th>
<th>blank</th>
<th>Reference calibrator (control)</th>
<th>Sample</th>
<th>Not solubilising control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation buffer</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant</td>
<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>ONPG Substrate</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Incubate 15 min at 37 °C in the dark

<table>
<thead>
<tr>
<th></th>
<th>blank</th>
<th>Reference calibrator (control)</th>
<th>Sample</th>
<th>Not solubilising control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop solution</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Read the absorbance against the blank at 420 nm (405/420)
9. RESULTS

9.1 Calculation of results
Calculate the mean absorbance of reference calibrator, controls and each sample.

The result can be expressed as
a. CH50 value
b. % of reference calibrator

Determinate the results using the following formula:

a. \( \frac{OD(\text{sample})}{OD(\text{Reference Calibrator})} \times \text{CH50 (value of Reference Calibrator)} = \text{CH50 Value of sample} \)
b. \( \frac{OD(\text{sample})}{OD(\text{Reference Calibrator})} \times \text{CH50 (% of Reference Calibrator)} = \% \text{ of Reference Calibrator} \)

Absorbance of reference calibrator
Example: CH50 value of reference calibrator vial = 100
CH50 % of reference calibrator vial = 50

Absorbance of reference calibrator = 0.350
Absorbance of sample = 1.108

a. CH50 value of sample = \( \frac{1.108}{0.350} \times 100 = 316 \)
b. % of reference calibrator = \( \frac{1.108}{0.350} \times 50 = 158 \% \)

9.2. Reference Value

<table>
<thead>
<tr>
<th>% of Reference</th>
<th>CH50 Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>0 – 100</td>
<td>Absence or low</td>
</tr>
<tr>
<td>51 – 150</td>
<td>101 – 300</td>
<td>Normal</td>
</tr>
<tr>
<td>&gt; 151</td>
<td>&gt; 301</td>
<td>High</td>
</tr>
</tbody>
</table>

10. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CH50 for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

11. PERFORMANCE AND CHARACTERISTICS

11.1. Correlation
22 samples from healthy blood donors were tested with the GenWay CH50 kit and with a similar commercially available kit. The results were processed by ROC curves analysed.

Sensitivity 100.0%
Specificity 94.4%
Overall agreement 95.5%

12. LIMITATIONS

The GenWay CH50 results are not diagnostic in themselves. Test results should be interpreted in conjunction with other laboratory tests as well as the clinical presentation of the patient. The GenWay CH50 kit will provide an assessment of the functional activity of total complement. This test can determine abnormal complement levels but cannot identify the abnormal component or components. Individual component abnormalities or abnormalities in the alternative pathway can exist despite a normal CH50.

The traditional method for the activity determination of complement is the method total haemolysis. The GenWay CH50 method is based on the capacity of complement to solubilise the immunocomplex. Both the classic activation and the terminal complement components are measured in this reaction. Total complement activity is usually abnormal if any component is defective. Assessment of CH50 is useful in screening for genetic deficiencies in the complement system and in monitoring the progress of patients with immunocomplex disease.
13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette patient samples and reference calibrator without splashing accurately to the bottom of microplate wells.

13.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. ORDERING INFORMATION

Prod. No.: 40-521-475033 CH 50 Determination (96 Determinations)

Additional reference serum is available separately
Reference serum (lyophilized)
BIBLIOGRAPHY


engl-11102010-CR