CERTEST

*Clostridium difficile* GDH+ Toxin A+B

ONE STEP
*Clostridium difficile* antigen GDH, Toxin A and Toxin B
COMBO CARD TEST

CERTEST BIOTEC S.L.
CERTEST *Clostridium difficile* GDH+Toxin A+B

**One Step test to detect *Clostridium difficile* antigen GDH, Toxin A and Toxin B in combo card format/Prueba combo de un solo paso para detección del antígeno GDH de *Clostridium difficile*, Toxina A y Toxina B en formato cassette**

**INTENDED USE**

CerTest *Clostridium difficile* GDH+Toxin A+B one step combo card test is a coloured chromatographic immunoassay for the simultaneous qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in stool samples. CerTest *Clostridium difficile* GDH+Toxin A+B combo card test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of *Clostridium difficile* infection.

**INTRODUCTION**

*Clostridium difficile* (*C. difficile*), a Gram-positive spore bearing anaerobic bacterium is the major aetiological agent of diarrhoea and colitis associated with antibiotics. *C. difficile* is the most common cause of health care-associated diarrhoea in developed countries and is a major source of nosocomial morbidity and mortality worldwide.

Disease due to *C. difficile* develops when the organism is allowed to proliferate in the colon, most commonly after antibiotic use has eliminated competing flora. *C. difficile* can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for the clinical manifestations, which range from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon, and death. *Clostridium difficile* Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

The toxigenic culture (TC) is used as the gold standard technique to determine *Clostridium difficile* infection. This method consists in culture and isolation of *C. difficile* from faeces, followed by toxin testing of the isolate, a labour-intensive assay to obtain a result.

**TEST PRINCIPLE**

CerTest *Clostridium difficile* GDH+Toxin A+B is based on the principle of a qualitative immunochromatographic assay for the determination of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in stool samples.

**Strip A** consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against GDH and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-GDH) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

**Strip B** consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against Toxin A and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Toxin A) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

**Strip C** consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against Toxin B and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Toxin B) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

If the sample is GDH positive, the antigen of the diluted sample reacts with the red-coloured conjugates complex (anti-GDH monoclonal antibodies-red polystyrene microspheres) in the strip A, if the sample is Toxin A positive, the antigens of the diluted sample reacts with the red-coloured conjugates complex (anti-Toxin A monoclonal antibodies-red polystyrene microspheres) in the strip B and if the sample is Toxin B positive, the antigens of the diluted sample react with the red-coloured conjugated complex (anti-Toxin B monoclonal antibodies-red polystyrene microspheres) in strip C, which were previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-GDH antibodies present on the membrane of strip A (test line), the anti-Toxin A antibodies present on the membrane of strip B (test line) and the anti-Toxin B antibodies present on the membrane of strip C (test line) capture the coloured conjugate and the red lines will be visible in the three strips. These bands are used to interpret the result.

If the sample is negative, there is no GDH, Toxin A and Toxin B presence and yet, the antigens may be present in a concentration lower than the detection limit value, for which the reaction will not take place with any red-coloured conjugate complex. The anti-GDH, the anti-Toxin A and the anti-Toxin B antibodies present on the membranes (test lines) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red lines will not appear.

Whether the sample is positive or not, in the three strips, the mixture continues to move across the membranes to the immobilized specific antibodies placed in the control lines. The anti-specific protein antibodies present on three membranes will capture control green-conjugate complex and three control lines will always appear. The presence of these green lines serve as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.
**STORAGE AND STABILITY**

Store as packaged in the sealed pouch at 2-30°C. The test is stable until the expiration date marked on its sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

**PRECAUTIONS**

- For professional *in vitro* diagnostic use.
- Do not use after expiration date.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. A new test must be used for each sample to avoid contamination errors.
- The tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use in the CerTest *Clostridium difficile* GDH+Toxin A+B combo card test. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink or smoke in the working area.

**SPECIMEN COLLECTION AND PREPARATION**

Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8°C) for 24 hours prior to testing. For longer storage, the specimen must be kept frozen at -20°C. Freezing and thawing cycles are not recommended. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenise stool sample as thoroughly as possible prior to preparation.

Specimen preparation (see illustration):
1. Take out the cap of the stool collection tube (1) and use the stick to pick up sufficient sample quantity. Then, introduce the stick once into 4 different parts of the stool sample (2), to collect faecal sample (approx. 125mg) and add it to the stool collection tube. For liquid samples, add approx. 125µL in the stool collection tube using a micropipette.
2. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion (3).

**MATERIALS**

**MATERIALS PROVIDED**
- CerTest *Clostridium difficile* GDH+Toxin A+B combo card tests
- Instructions for use
- Stool collection tubes with diluent

**MATERIALS REQUIRED BUT NOT PROVIDED**
- Specimen collection container
- Disposable gloves
- Timer

**TEST PROCEDURE**

Allow tests, stool samples and controls to reach room temperature (15-30°C) prior to testing. Do not open pouches until the performance of the assay.

1. Proceed to shake the stool collection tube in order to assure good sample dispersion.
2. Remove CerTest *Clostridium difficile* GDH+Toxin A+B combo card test from its sealed bag just before using it.
3. Take the stool collection tube, cut the end of the cap (4) and dispense 4 drops in the circular window marked with the letter A (5), 4 drops, using the same tube, in the circular window marked with the letter B (6), and 4 drops, using the same tube, in the circular window marked with the letter C (7). Avoid adding solid particles with the liquid.
4. Read the results at 10 minutes. Do not read the test result later than 10 minutes.
CERTEST Clostridium difficile GDH+Toxin A+B
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If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn’t work, dispense a drop of extraction diluent until seeing the liquid running through the reaction zone.

INTERPRETATION OF THE RESULTS (please refer to the illustration below)

1. A: Green ➔ Negative GDH
   B: Green ➔ Negative Toxin A
   C: Green ➔ Negative Toxin B

2. A: Green/Red ➔ Positive GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

3. A: Green/Red ➔ Positive GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

4. A: Green/Red ➔ Positive GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

5. A: Green/Red ➔ Positive GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

6. A: Green ➔ Negative GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

7. A: Green ➔ Negative GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

8. A: Green ➔ Negative GDH
   B: Green/Red ➔ Positive Toxin A
   C: Green/Red ➔ Positive Toxin B

OTHER RESULTS

- Add 4 drops in circular window (A)
- Add 4 drops in circular window (B)
- Add 4 drops in circular window (C)
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<table>
<thead>
<tr>
<th>A (GDH)</th>
<th>B (Toxin A)</th>
<th>C (Toxin B)</th>
<th>Interpretation of the results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-</td>
<td>-</td>
<td>There are no GDH, Toxin A and Toxin B of Clostridium difficile. No infection caused by Clostridium difficile.</td>
</tr>
<tr>
<td></td>
<td>GREEN</td>
<td>GREEN</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>+</td>
<td>+</td>
<td>There are GDH, Toxin A and Toxin B of Clostridium difficile presence. Infection caused by Clostridium difficile.</td>
</tr>
<tr>
<td></td>
<td>GREEN-RED</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>+</td>
<td>+</td>
<td>There is GDH and Toxin A presence. Infection caused by Clostridium difficile.</td>
</tr>
<tr>
<td></td>
<td>GREEN-RED</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>+</td>
<td>-</td>
<td>There is GDH and Toxin B presence. Infection caused by Clostridium difficile.</td>
</tr>
<tr>
<td></td>
<td>GREEN-RED</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>+</td>
<td>-</td>
<td>There is GDH presence. Infection caused by Clostridium difficile.</td>
</tr>
<tr>
<td></td>
<td>GREEN-RED</td>
<td>GREEN</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>-</td>
<td>+</td>
<td>If this result appears it must be repeat the test using a fresh sample. If results are again positive for Toxin A and B and negative for GDH, the sample should be considered positive for Toxin A and B.</td>
</tr>
<tr>
<td></td>
<td>GREEN</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>-</td>
<td>+</td>
<td>If this result appears it must be repeat the test using a fresh sample. If the result is again positive for Toxin A and negative for GDH, the sample should be considered positive for Toxin A.</td>
</tr>
<tr>
<td></td>
<td>GREEN</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>-</td>
<td>-</td>
<td>If this result appears it must be repeat the test using a fresh sample. If the result is again positive for Toxin B and negative for GDH, the sample should be considered positive for Toxin B.</td>
</tr>
<tr>
<td></td>
<td>GREEN</td>
<td>GREEN-RED</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Any other result</td>
<td>Invalid result either A, B or C, we recommend repeating the assay using the same sample with another test.</td>
<td></td>
</tr>
</tbody>
</table>

6, 7 and 8, a very low percentage of specimens might result negative for GDH but positive for toxins.  

INVALID: Total absence of any control coloured line (GREEN) regardless the appearance or not of the test lines (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

NOTES ON THE INTERPRETATION OF RESULTS
The intensity of the red coloured bands in the test lines (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

QUALITY CONTROL
Internal procedural controls are included in the test. The green lines appearing in the control lines (C) in the results windows are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS
1. The test must be carried out within 2 hours after opening the sealed bag.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
3. The intensity of test line may vary from very strong at high antigens concentration to faint when the antigens concentration is close to the detection limit value of the test.
4. CerTest Clostridium difficile GDH+Toxin A+B should be used only with samples from human faeces. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
5. Positive results determine the presence of GDH, Toxin A and Toxin B of Clostridium difficile in faecal samples. A positive result should be followed up with additional laboratory techniques (toxigenic culture) to determine the strain. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
6. A negative result is not meaningful because of it is possible the antigens concentration in the stool samples is lower than the detection limit value. If the symptoms or situation still persist, a Clostridium difficile determination should be carried out on a sample from an enrichment culture.
EXPECTED VALUES

Clostridium difficile is associated with 95-100% cases of pseudomembranous colitis, 60-75% cases of antibiotic-associated colitis and 35% of cases of antibiotic-associated diarrhoea cases.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit values of CerTest Clostridium difficile GDH+Toxin A+B are 0.8ng/mL for GDH, 2ng/mL for Toxin A and 0.63ng/mL for Toxin B.

Clinical sensitivity and specificity (GDH)

An evaluation was performed comparing two immunochromatographic tests to detect Clostridium difficile infection (CerTest Clostridium difficile GDH+Toxin A+B, CerTest vs. C. DIFF QUIK CHEK Complete®, Techlab). The samples were directly taken from patients suffering diarrhoea. Positive samples were confirmed with ELISA assay. The results were as follows:

<table>
<thead>
<tr>
<th>IC test: CerTest Clostridium difficile GDH+Toxin A+B (GDH)</th>
<th>IC test: C. DIFF QUIK CHEK Complete®</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CerTest Clostridium difficile GDH+Toxin A+B (GDH) vs C. DIFF QUIK CHEK Complete®</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

Another evaluation was performed using positive and negative samples comparing IC test (CerTest Clostridium difficile GDH+Toxin A+B, CerTest) to an ELISA assay (Wampole™ C. Diff Chek™-60, Techlab). The results were as follows:

<table>
<thead>
<tr>
<th>IC test: CerTest Clostridium difficile GDH+Toxin A+B (GDH)</th>
<th>EIA test: Wampole™ C. Diff Chek™-60</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CerTest Clostridium difficile GDH+Toxin A+B (GDH) vs Wampole™ C. Diff Chek™-60</td>
<td>&gt;95%</td>
<td>&gt;99%</td>
<td>&gt;99% 96%</td>
</tr>
</tbody>
</table>

Clinical sensitivity and specificity (Toxin A-B)

An evaluation was performed comparing two immunochromatographic tests to detect Toxin A and Toxin B of Clostridium difficile infection (CerTest Clostridium difficile GDH+Toxin A+B, CerTest vs. C. DIFF QUIK CHEK Complete®, Techlab). The samples were directly taken from patients suffering diarrhoea. Positive samples were confirmed with ELISA assay. The results were as follows:
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The results showed a high sensitivity and specificity to detect GDH, Toxin A and Toxin B of Clostridium difficile using CerTest Clostridium difficile GDH+Toxin A+B.

Cross reactivity
An evaluation was performed to determine the cross reactivity of CerTest Clostridium difficile GDH+Toxin A+B; no cross reactivity against gastrointestinal pathogens occasionally present in faeces:

<table>
<thead>
<tr>
<th>CerTest Clostridium difficile GDH+Toxin A+B (Toxin A-B) vs C. DIFF QUIK CHEK Complete®</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Campylobacter coli</th>
<th>Salmonella enteritidis</th>
<th>Shigella dysenteriae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>Salmonella paratyphi</td>
<td>Shigella flexneri</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>Salmonella typhi</td>
<td>Shigella sonnei</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Salmonella typhimurium</td>
<td>Staphilococcus aureus</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Shigella boydii</td>
<td>Yersinia enterocolitica</td>
</tr>
</tbody>
</table>
REFERENCES/BIBLIOGRAFÍA


SYMBOLS FOR IVD COMPONENTS AND REAGENTS/SÍMBOLOS PARA REACTIVOS Y PRODUCTOS PARA DIAGNÓSTICO IN VITRO

- In vitro diagnostic device: Producto para diagnóstico in vitro
- Keep dry: Almacenar en lugar seco
- Use by: Fecha de caducidad
- Manufacturer: Fabricante
- Temperature limitation: Limitación de temperatura
- Contains sufficient for <n> test: Contiene <n> test
- Sample diluent: Diluyente de muestra
- Catalogue number: Número de referencia
- Batch code: Número de lote