CLOSTRIDIUM TOXIN A+B CARD

Immunochromatographic rapid test
for the detection of Clostridium Difficile Toxin A and Toxin B in feces

EXPLANATION OF THE TEST
Clostridium difficile is a major cause of antibiotic-associated
diarrhea and pseudomembranous colitis (1). It is now one of
the most commonly detected pathogens and an important cause
of nosocomial infections in hospitals and nursing homes (2, 3).
The organism has been isolated from diverse natural habitats,
including soils, hay, sand, dung from various large mammals
(cows, donkeys and horses), and from dog, cat, rodent and
human feces (4). C.difficile produces at least three potential
virulence factors from which Toxin A and Toxin B are thought
to be the most important in the pathogenesis of C.difficile
associated diseases (5).

Toxin A is an enterotoxin which seems to interfere with the
cytoskeleton of the intestinal epithelial cells, rendering them
non functional while Toxin B is a cytotoxin that induces strong
cytopathic effects in tissue cultures cell lines (6).

Since not all strains of Clostridium difficile produce toxins and
approx. 2% of healthy adults as well as up to 50% of children
younger than 2 years can be colonized with Clostridium
difficile, the detection of the toxins (Toxin A and Toxin B) in
stool samples of patients with diarrhea is more significant than
culturing the bacteria.

The CLOSTRIDIUM TOXIN A+B is a lateral flow,
immunochromatographic rapid test for the qualitative detection
of Clostridium difficile Toxin A and Toxin B in human feces.
The test device consists of a plastic housing containing two
different sticks for the detection of C.difficile Toxin A or
C.difficile Toxin B. A coloured anti C.difficile Toxin
C.difficile Toxin B monoclonal antibody colloidal
conjugate is placed at the left end of the membrane.

1)Preliminary notes
Stool specimen should be collected as soon as possible after
onset of symptoms.

Diluted samples may be stored at +2°C to +8°C for 3 days
without interference with assay performance.

For long term storage of undiluted specimens, storage at –20°C
or colder is recommended. Repeated freezing and thawing of
samples is not recommended and may cause erroneous results

Caution!
Do not collect specimens in containers having media,
preservatives, animal serum or detergents, as any of these
may interfere with the test.

2) Procedure
1 - Write the name of the patient or a control number and date
   on the label of the sample collection device.

2 - Unscrew the top of the sample collection device containing
   sample collection probe.

3 - Collect the feces sample with the top of the collection device
   deeping it in 3 different places of the same feces sample and
   put it in the collection device. The feces volume added into the
diluent should be approximately the volume of a pea (in case of
solid feces). If the feces are liquid, the volume should be
around 200 µL.

4 - Return the sample collection probe loaded with the sample in
   its place on collection device and screw it firmly.

PRECAUTIONS
1 - This test is designed for in vitro diagnostic use and professional
   use only.
2 - Read carefully instructions leaflet before using this test.
3 - Do not use beyond the expiry date stated on the package label.
4 - Do not use a test from a damaged protective wrapper.
5 - All reagents and materials coming in contact with potential
   infectious specimens must be treated with appropriate
   disinfectants
   or autoclaved at 121°C for at least one hour.
6 - Wear protective clothing such as laboratory coats and
disposable gloves while assaying samples.
7 - Do not eat, drink or smoke in the area where specimens and kit
   reagents are handled.
8 - Avoid any contact between hands and eyes or nose during
   specimen collection and testing.

STORAGE AND STABILITY
1 - All CLOSTRIDIUM TOXIN A+B kit components should
   be stored in the sealed pouch at room temperature (between
   +4°C and +30°C).
2 - Do not freeze the test kit.
3 - The CLOSTRIDIUM TOXIN A+B-CHECK-1 kit is stable until
   the expiry date stated on the package label.

SPECIMEN COLLECTION AND PREPARATION

MATERIALS PROVIDED
Each kit contains everything needed to perform 25 tests.
- CLOSTRIDIUM TOXIN A+B reaction devices 25
- Sample collection devices containing 2 mL of solution 25
- Instruction leaflet 1
- Positive control (optional): A freeze-dried
  optionally available as a positive control
  preparation is It should be reconstituted with 0.6 mL of extraction
  solution and produces an assay result equivalent to that produced
  by positive specimens (i.e. pink color) and should be kept at +2°C
to +8°C after reconstitution.1
PROCEDURE OF THE TEST

a) Samples
1. Bring all reagents to come to room temperature before testing.
2. Remove the test device from the pouch.
3. Break the tip of the sample collection device and add 6 drops of extracted sample into each sample well (D) on the reaction device.
4. Read the results of the test 10 minutes after addition of the sample on the device.

b) Positive control (optional)
1. Remove the test device from the pouch.
2. Add 200 µL of the positive control into the sample well on reaction device.
3. Read the results of the test after 15 minutes.

INTERPRETATION OF THE TEST

POSSIBLE RESULTS PATTERN

| TOX. A + | TOX. B - |
| TOX. A - | TOX. B + |

1. Negative
Only one coloured band appears in the control zone. No band is visible in the test zone.

2. Positive
In addition to the control band, a clearly distinguishable band also appears in the test zone.

3. Inconclusive
If there is no distinct coloured band visible in the control zone, the test is inconclusive. Repeat the test.

PERFORMANCES

1. C. difficile TOXIN A
   a) Analytical sensitivity
   The performance of the test has been assayed, using a range of diluted solutions prepared from a commercially available purified toxin A antigen. The test is able to detect a concentration of 4 ng/mL.
   b) Diagnostic sensitivity and specificity
   A study was performed on 63 feces samples, using the TOXIN A-CHECK-1 test in comparison with the cytotoxicity and cell culture methods.
   The results are summarized in the table 1:

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>4</td>
<td>44</td>
<td>48</td>
</tr>
<tr>
<td>+</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Evaluation results

All samples showed identical results using the cytotoxicity and the cell culture methods.

From the above table, the sensitivity of TOXIN A test is 86.7% (13/15), compared to the cytotoxicity method and the specificity is 91.7% (44/48), compared to the cytotoxicity method.

2. C. difficile TOXIN B
   a) Analytical sensitivity
   The sensitivity of the test has been evaluated using a range of diluted solutions prepared from a commercially available purified C.D. Toxin B antigen. Under these conditions, the detection limit of the test has been found to be 5 ng/mL.
   b) Specificity
   A purified C.D. Toxin A antigen was used to determine the specificity of the test.
   TOXIN B-CHECK-1 showed consistently negative results up to 500 ng/mL. TOXIN A.

LIMITATIONS

1. The CLOSTRIDIUM TOXIN A+B
   is specifically designed to detect Toxin A or Toxin B antigen in the stool samples.

2. As for any in vitro diagnostic procedure, the physician should confirm the test results with other clinical methods.

3. A negative result does not generally exclude a C. difficile infection. It can be caused by proteolytic digestion of the toxins due to improper specimen storage. If a reasonable suspicion of an infection exists, another stool specimen should be investigated.

4. A positive result does not exclude the presence of other pathogens.

5. Test and control lines colours may slightly change depending on the stool sample aspect. For example dark green lines (instead of pink lines) have been reported when assaying greenish or darkish stool samples. This stool coloration appears in case of treatment of iron deficiency with ferrous fumarate. The test result should be interpreted as usual, i.e. two lines for a positive result and one line for a negative result.

REFERENCES


Phone: +39 (0)813302705 / +39 (0)815063417
Fax: +39 (0)813301453
Website: www.intermedical.it
E-mail: specialist@intermedical.it


**GRAPHICAL SYMBOLS USED**

<table>
<thead>
<tr>
<th>Icon</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Storage temperature" /></td>
<td>Storage temperature</td>
</tr>
<tr>
<td><img src="image" alt="LOT" /></td>
<td>Lot number</td>
</tr>
<tr>
<td><img src="image" alt="In vitro diagnostic device" /></td>
<td>In vitro diagnostic device</td>
</tr>
<tr>
<td><img src="image" alt="Expiry date" /></td>
<td>Expiry date</td>
</tr>
<tr>
<td><img src="image" alt="Catalogue number" /></td>
<td>Catalogue number</td>
</tr>
<tr>
<td><img src="image" alt="Determination number for kit" /></td>
<td>Determination number for kit</td>
</tr>
<tr>
<td><img src="image" alt="Read instruction before use" /></td>
<td>Read instruction before use</td>
</tr>
<tr>
<td><img src="image" alt="Manufacturer" /></td>
<td>Manufacturer</td>
</tr>
</tbody>
</table>

Rev. 1 09/2018