CLOSTRIDIUM TRIO TOXIN A/B/GDH
Immunochromatographic rapid test
for the detection of Clostridium Difficile Toxin A, Toxin B and GDH in feces

Ref. C-69T

I- PRINCIPLE

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), 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). Known as the common C. difficile antigen, Glutamate Dehydrogenase (GDH) is a metabolic enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for organism (6,7).

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, Toxin B and GDH) in stool samples of patients with diarrhea is more significant than culturing the bacteria.

The TRIO TOXIN A/B/GDH is a lateral flow, immunochromatographic rapid test for the qualitative detection of Clostridium difficile Toxin A, Toxin B and GDH in human feces.

The test device consists of a plastic housing containing three different sticks for the detection of C. difficile Toxin A , C. difficile Toxin B or C. difficile GDH. A coloured anti C. difficile Toxin A, C. difficile Toxin B and C. difficile GDH monoclonal antibody colloidal gold conjugate is placed at the left end of the membrane.

After collection in a tube containing the extraction solution, the feces sample is dissolved and few drops of this extract are added into each well ( ) of the reaction device.

As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the Toxin A, Toxin B or GDH antigen (when present in the sample), forming an antibody antigen complex.

This complex binds to the anti-Toxin A, Toxin B and GDH antibody in the positive reaction zone, producing a rose-pink coloured band.

In the absence of Toxin A, Toxin B or GDH, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device, past the positive reaction zone and control zone.

Unbound conjugate binds to the reagent in the control zone producing a rose-pink coloured band demonstrating that the reagents are functioning correctly.

II- TRIO TOXIN A/B/GDH- KIT COMPONENTS

Each kit contains everything needed to perform 20 tests.

- TRIO TOXIN A/B/GDH reaction devices 20
- Sample collection devices containing 2 mL of solution (bottle type) 20
- Instructions leaflet 1
- Positive control for CD Tox A and CD Tox B only (optional): A freeze-dried preparation is optionally available as a positive control. 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 colour) and should be kept at +2°C to +8°C after reconstitution.

III- STORAGE AND STABILITY

1- All TRIO TOXIN A/B/GDH 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 TRIO TOXIN A/B/GDH kit is stable until the expiry date stated on the package label.

IV- 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.

V- SPECIMEN COLLECTION AND PREPARATION

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- Indicate the name of the patient or a control number as well as date on the 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.

VI- ASSAY PROCEDURE

1) 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 3 drops (100µL) of extracted sample into each sample well on the reaction device.
4- Read the results of the test 10 minutes after addition of the sample on the device.

2) Positive control for CD Tox A and CD Tox B only (optional)

1- Remove the test device from the pouch
2- Add 100 µL of the positive control into the sample well on reaction device.

VII- READING TEST RESULTS

POSSIBLE RESULTS PATTERN

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.

VIII- PERFORMANCES CHARACTERISTICS

1) GDH

a) Analytical sensitivity
The performance of the test has been evaluated using a range of diluted solutions prepared from a commercially available purified GDH antigen. The test is able to detect a concentration of 5 ng/mL. A competitor GDH test showed an analytical sensitivity of 10 ng/mL when the same GDH antigen was assayed.

b) Diagnostic sensitivity and specificity
A comparative study between Diasorin analyser and GDH rapid test has been performed using a panel of 59 human stool samples.

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<tr>
<th></th>
<th>DIASORIN GDH (reference method)</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GDH</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>40</td>
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Table 2: GDH versus Diasorin GDH results
From the above table, the diagnostic sensitivity of GDH test is 81% (13/16) and the specificity is 97.5% (39/40) when compared to the reference method. Overall agreement is 93% (52/56). Two samples were identified as equivocal by Diasorin analyzer and one sample was found doubtful by GDH. Therefore they were not included in the calculation.

c) Cross reaction
The following organisms were tested and did not show any cross reaction:

- Campylobacter spp
- Escherichia coli
- Helicobacter pylori
- Listeria mononytogenes
- Salmonella spp
- Shigella spp
- Staphilococcus aureus
- Yersinia enterocolitica
- *C. Difficile* Toxin A
- *C. Difficile* Toxin B
- Rotavirus
- Adenovirus

d) Intra assay reproducibility
The in-vitro reproducibility was evaluated by running 15 replicates of three samples dilution having different GDH concentration. All the series showed converging results in conformance with the expected results. Intra-assay reproducibility performance of GDH is 100%.

2) *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 test in comparison with the cytotoxicity and cell culture methods. The results are summarized in the table 1:

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
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<th>Total</th>
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<tr>
<td>+</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>63</td>
</tr>
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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.

c) Cross reaction
TOXIN A showed consistently negative results up to 500 ng/mL Toxin B.

3) *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 showed consistently negative results up to 500 ng/mL Toxin A.

IX - LIMITATIONS

1- The TRIO TOXIN A/B/GDH is specifically designed to detect Toxin A, Toxin B or GDH 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.

X- BIBLIOGRAPHY


13-Clostridium difficile : toxin testing alone is not enough. (August 2008). The Biomedical Scientist 689-691.

Temperature limitations

Consult operating instructions

Do not re-use

In vitro diagnostic use

Manufactured by