Clostridium difficile GDH Rapid Test (Feces)
For professional use

CE IVD REF I018-6002

In vitro rapid diagnostic test for the detection of Clostridium difficile GDH antigen in human feces samples.
For in vitro use. For professional use only.

INTENDED USE

The *Clostridium difficile* GDH Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of *Clostridium difficile* GDH antigen in the human feces specimen.

SUMMARY

Clostridium difficile is an anaerobic bacteria acting as an opportunistic pathogen: it grows in the intestine when the normal flora has been altered by treatment with antibiotics.[1,2,3] Toxinogenic strains of *Clostridium difficile* cause infections from mild-diarrhea to pseudomembranous colitis, potentially leading to death.[4]
Disease is caused by two toxins produced by toxinogenic strains of C.difficile: Toxin A (tissue-damaging enterotoxin) and Toxin B (cytotoxin). Some strains produce both toxins A and B, some others produce Toxin B only. The potential role of a third (binary) toxin in pathogenicity is still debated.[4]
The use of Glutamate Dehydrogenase (GDH) as an antigen marker of *C.difficile* proliferation has been shown to be very effective because all strains produce high amount of this enzyme.[5,6]
Clostridium difficile GDH Rapid Test allows the specific detection of *C. difficile's* GDH in stool specimen. Samples with a positive result should be investigated further to test for toxigenicity of the bacteria.

PRINCIPLES

This is a ready-to-use test that is based on the use of a membrane technology with colloidal gold. A nitrocellulose membrane is sensitized with antibody directed against *Clostridium difficile* antigen (GDH). The test's specificity is ensured by an antibody specific to the *Clostridium difficile* GDH that is conjugated to the colloidal gold. This conjugate is dried on polyester.
The fecal sample must be diluted into the extraction buffer that is supplied with the test. When the fecal suspension come into contact with the strip, the solubilized conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the anti-Clostridium antibody adsorbed on to the nitrocellulose. If the sample contains *C. difficile* GDH, the conjugate-antigen complex will remain bound to the anti-*C. difficile* GDH reagent and a red line will develop. Solution continues to migrate to encounter a second reagent that binds the migration control conjugate, thereby producing a red control line that confirms that the test is working properly. The result is visible within 10 minutes.

MATERIALS

Materials provided

- Test cassettes
- Droppers
- Specimen collection tubes with buffer
- Package insert

Materials required but not provided

- Timer
- Specimen collection containers

WARNINGS AND PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- All reagents are for *in vitro* diagnostic use only.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Reagents' quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in this insert.
- Dispose of gloves, swabs, test tubes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and mustensure that it is disposed of in accordance with the applicable legislation.

STORAGE AND STABILITY

Store as packaged at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

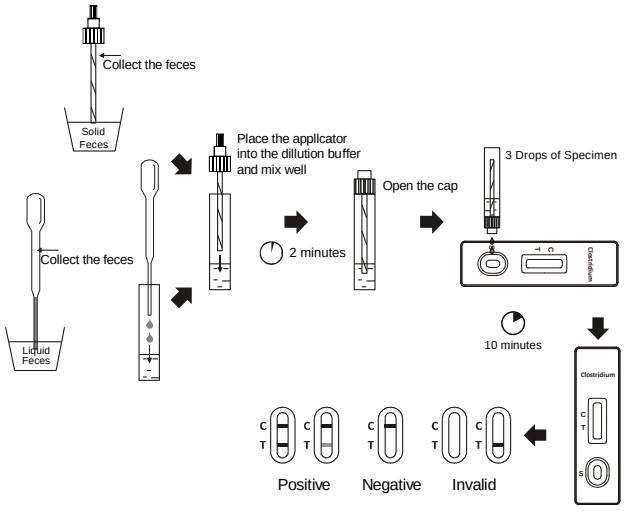
The stool specimens must be tested as soon as possible after collection. If necessary, original feces specimen could be stored at 2-8°C for 3 days or -20°C for longer periods of time; extracted specimen in buffer could be stored at 2-8°C for 1 week or -20°C for longer periods of time.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.

DIRECTIONS FOR USE

Allow the test, specimen, stool collection buffer and/or control to equilibrate to room temperature (15-30°C) prior to testing.

1. To process fecal specimens:
   - For **Solid Specimens**:
     Unscrew the cap of the specimen collection tube, then randomly **stab the specimen collection applicator into the fecal specimen at least 3 different sites** to collect approximately **50 mg of feces** (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.
   - For **Liquid Specimens**:
     Hold the dropper vertically, aspirate fecal specimens, and then transfer **2 drops of the liquid specimen** (approximately 80 µL) into the specimen collection tube containing the extraction buffer.

   Tighten the cap onto the specimen collection tube, then **shake the specimen collection tube vigorously** to mix the specimen and the extraction buffer. Leave the collection tube for reaction for 2 minutes.
2. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.
3. Hold the specimen collection tube upright and **unscrew the cap** of the specimen collection tube. Invert the specimen collection tube and **transfer 3 full drops of the extracted specimen** (approximately 120 µL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
4. Read the results at **10 minutes** after dispensing the specimen. Do not read results after 20 minutes.
5. **Note:** If the specimen does not migrate (presence of particles), centrifuge the diluted sample contained in the extraction buffer vial. Collect 80 µL of supernatant, dispense into the specimen well (S).of a new test cassette and start afresh following the instructions mentioned above.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE:* **Two colored lines appear.** One colored line should be in the control line region (C) and another colored line should be in the test line region (T).
***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of *Clostridium difficile GDH* antigen present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.
NEGATIVE: One colored line appears in the control line region (C). No line appears in the test line region (T).
INVALID: Control line (C) fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor
Note: during the drying process, a very faint shadow may appear at the test line position. It should not be regarded as a positive result.

QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis.
2. A positive test does not rule out the possibility that other pathogens may be present.

PERFORMANCE CHARACTERISTICS

Detection Limit

The detection limit was evaluated by diluting a purified GDH preparation and the results show that the concentration of protein detected is 1ng/mL.

Sensitivity - Specificity

Method		Other Rapid Test		Total Results
Clostridium difficile GDH Rapid Test (Feces)	Results	Positive	Negative	
	Positive	78	2	80
	Negative	1	119	120
Total Results		79	121	200

Relative Sensitivity: 98.7% (95%CI:*93.1%-100%)
Relative Specificity: 98.3% (95%CI:*94.2%-99.8%)
Relative Accuracy: 98.5% (95%CI:*95.7%-99.7%) *Confidence Intervals

Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected. To check inter-batch accuracy (reproducibility), some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

Cross Reactivity

An evaluation was performed to determine the cross reactivity of *Clostridium difficile* GDH Rapid Test (Feces). No cross reactivity against gastrointestinal pathogens occasionally present as following:

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
E.coli O157:H7	*Salmonella typhi*	*Shigella sonnei*
H.pylori	*Salmonella typhimurium*	*Staphylococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

BIBLIOGRAPHY

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INDEX OF SYMBOLS

	Consult instructions for use		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by date
	Temperature limit	LOT	Batch code
	Do not use if package is damaged		Manufacturer
EC REP	Authorized representative in the european community		Do not re-use
REF	Catalogue number		

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EC REP
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