Clostridium difficile Toxin A+Toxin B Combo Rapid Test (Feces)
For professional use

CE IVD REF I019-6025B

A rapid test for the detection of Clostridium difficile Toxin A and Toxin B antigens in human feces samples.
For in vitro professional use only.

INTENDED USE

The *Clostridium difficile* Toxin A+Toxin B Combo Rapid Test (Feces) is a rapid chromatographic immunoassay for the qualitative detection of *Clostridium difficile* Toxin A and Toxin B antigens 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]

PRINCIPLES

Clostridium difficile Toxin A+Toxin B Combo Rapid Test (Feces) detects two distinct antigens in fecal specimens for *C. difficile*, *viz.*, Toxin A and Toxin B on two different test strips in a single test devices, thus simultaneously detecting two antigens specific to *Clostridium difficile*.

For C.difficile-specific Toxin A Testing

The membrane is precoated with anti-C.diff Toxin A antibody and anti-C.diff Toxin A antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-C.diff Toxin A antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.diff Toxin A antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

For C.difficile-specifc Toxin B Testing

The membrane is precoated with anti-C.diff Toxin B antibody and anti-C.diff Toxin B antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-C.diff Toxin B antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.diff Toxin B antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

Materials provided

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

Materials required but not provided

- Timer
- Stool containers
- Centrifuge

WARNINGS AND PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

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 may be stored at 2-8°C for 3 days or -20°C for longer periods of time; extracted specimen in buffer may 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.

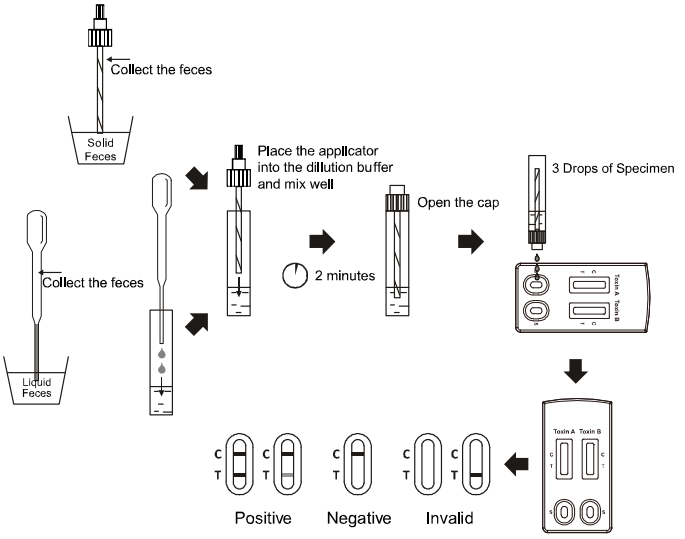
DIRECTIONS FOR USE

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

1. To collect fecal specimens:
   Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain enough antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long term storage, specimens should be kept below -20°C.
2. 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, and then **shake the specimen collection tube vigorously** to mix the specimen and the extraction buffer. Leave the collection tube for reaction for 2 minutes.
3. Bring the pouch to room temperature before opening it. Remove the test devices 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.
4. Hold the specimen collection tube upright and **unscrew the tip** of the specimen collection tube. Invert the specimen collection tube and **transfer 3 full drops of the extracted specimen** (approximately 120 µL) to each of the specimen well (S) of the test devices, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
5. Read the results at **10 minutes** after dispensing the specimen. Do not read results after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the diluted sample contained in the extraction buffer vial. Collect 120 µL of supernatant, dispense into the specimen well (S). Start the timer and continue from step 5 onwards in the above instructions for use.



INTERPRETATION OF RESULTS

The test results appear in two different test windows respectively for Toxin A or Toxin B. The interpretation criteria remain the same for positivity or negativity for specific antigens under tests as per indication of the respective Test window. The results are to be interpreted as follows:
POSITIVE:*Two colored lines appear. One colored line should be in the control line region (C) and another apparent 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 antigens 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. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal positive 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 *Clostridium difficile* Toxin A+Toxin B Combo Rapid Test (Feces) is for *in vitro* diagnostic use only.
2. 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.
3. A positive test does not rule out the possibility that other pathogens may be present.

PERFORMANCE CHARACTERISTICS

Detection Limit

Detection limit values of *Clostridium difficile* Toxin A+Toxin B Combo Rapid Test (Feces) was 2 ng/mL for Toxin A and 7 ng/mL for Toxin B.

Sensitivity – Specificity

***Clostridium difficile* Toxin A Results**

Method		Other Rapid Test		Total Results
Clostridium difficile **Toxin A+Toxin B Rapid Test (Feces)**	**Results**	Positive	Negative	
	Positive	115	5	120
	Negative	7	173	180
Total Results		122	178	300

Relative Sensitivity: 94.3% (95%CI:*88.5%-97.7%) *Confidence Intervals
Relative Specificity: 97.2% (95%CI:*93.6%-99.1%)
Relative Accuracy: 96.0% (95%CI:*93.1%-97.9%)

***Clostridium difficile* Toxin B Results**

Method		Other Rapid Test		Total Results
Clostridium difficile **Toxin A+Toxin B Rapid Test (Feces)**	**Results**	Positive	Negative	
	Positive	112	6	118
	Negative	10	172	182
Total Results		122	178	300

Relative Sensitivity: 91.8% (95%CI:*85.4%-96.0%) *Confidence Intervals
Relative Specificity: 96.6% (95%CI:*92.8%-98.8%)
Relative Accuracy: 94.7% (95%CI:*91.5%-96.9%)

Precision

Intra-assay and inter-assay

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 3 times on test kits of the same batch number in the same

experimental conditions. All observed results were confirmed as expected.
To check inter-batch accuracy (reproducibility), same samples (positive and buffer) were processed on test kits from three different batches. All results were confirmed as expected.

Cross Reactivity

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

Campylobactercoli	*Salmonella enteritidis*	*Shigelladysenteriae*
Campylobacterjejuni	*Salmonella paratyphi*	*Shigellaflexneri*
E.coli O157:H7	*Salmonella typhi*	*Shigellasonnei*
H.pylori	*Salmonella typhimurium*	*Staphylococcus aureus*
Listeria monocytogenes	*Shigellaboydii*	*Yersiniaenterocolitica*

Interfering Substances

The following potentially Interfering Substances were added to *Clostridium difficile* Toxin A+Toxin B negative and positive specimens.

Ascoribic acid: 20 mg/dL	Oxalic acid: 60 mg/dL	Bilirubin: 100 mg/dL
Uric acid: 60 mg/dL	Aspirin: 20 mg/dL	Urea: 2000 mg/dL
Glucose: 2000 mg/dL	Caffeine: 40 mg/dL	Albumin: 2000 mg/dL

BIBLIOGRAPHY

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3. Leyerly D.M., H.C. Krivan and D.T.Wilkins: Clostridium difficile: its disease and toxins.Clinical Microbiology Reviews, p. 1-18, Jan. 1988
4. Ramsey L. et al: Fulminant Clostridium difficile: an underappreciated and increasing causeof death and complications, Annals of Surgery 235 (3) p. 363-372: Mar. 2002

INDEX OF SYMBOLS

	Consult instructions for use	Σ	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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