CDF-F42

C. difficile Toxin A/B Rapid Test Device (Feces)

INTENDED USE

The *C. difficile* Toxin A/B Rapid Test Device is a rapid chromatographic immunoassay for the simultaneous qualitative detection of *Clostridium difficile* Toxin A and Toxin B in human feces. It is intended to aid in the diagnosis and differentiation of *C. difficile* toxin A and toxin B infections. The test is for professional use only.

INTRODUCTION

C. difficile is a gram-positive, spore-forming anaerobic bacillus. Toxigenic strains of *C. difficile* produce two different toxins constituting the essential virulence factors for *C. difficile* infection (CDI). CDI is considered responsible for approximately 25% of the diarrhea incidents related to the consumption of antibiotics, and CDI can lead to pseudomembranous colitis (PMC), requiring urgent treatment with antibiotics effective against *C. difficile* and which, without treatment, may severely compromise the life of patients. In the case of PMC, CDI mortality can be as high as 6% to 30%[1,2].

Asymptomatic population may be carriers of toxigenic *C. difficile*, among which, some may be epidemic strains. Diagnosis and differentiation of *C. difficile* toxin A and toxin B play essential role with regard to both rapid treatment and disease transmission control[3].

Current diagnostic methods of CDI are mostly qualitative detection of the bacteria, toxins, or toxin genes. Rapid immunoassay of CDI has become more important due to the availability of direct diagnosis and effective treatment. Rapid diagnosis of CDI can lead to reduced hospital stays and cost of hospital care.

The *C. difficile* Toxin A/B Rapid Test Device is a lateral flow immunoassay using highly sensitive monoclonal antibodies that are specific for *C. difficile* toxin A and toxin B antigens. The test provides a simple, fast and separate detection of toxin A and toxin B in a single test.

PRINCIPLE

The *C. difficile* Toxin A/B Rapid Test Device detects *C. difficile* toxin A and toxin B antigens through visual interpretation of color development. The test device contains two separate strips inside one housing device with two separate windows. Anti-toxin A and anti-toxin B antibodies are immobilized on the test regions of the nitrocellulose membranes of two separate strips, respectively. A fecal sample is added to the sample diluent buffer which is optimized to extract the toxin A and/or B antigens from specimen. During testing, the extracted antigens, if present, will bind to anti-toxin A or anti-toxin B antibodies conjugated to colored particles on the sample pad of A or B strip. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by either anti-toxin A or anti-toxin B antibodies at the detection zone of each strip. Excess colored particles are captured at the internal control zone.

The presence of a red band in the A/B region indicates a positive result for the particular toxin antigens, while its absence indicates a negative result. A red band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

MATERIALS

Materials Provided

- Individually packed test devices
- Droppers
- Sample dilution tube with buffer
- Package insert

Materials Required but Not provided

- Specimen collection container
- Centrifuge
- Clock, timer or stopwatch
- Disposable latex gloves

PRECAUTIONS

- For in vitro Diagnostic Use Only.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
- Do not use the Sample diluent buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Care should be taken to store specimens as indicated in the document.
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.

STORAGE AND STABILITY

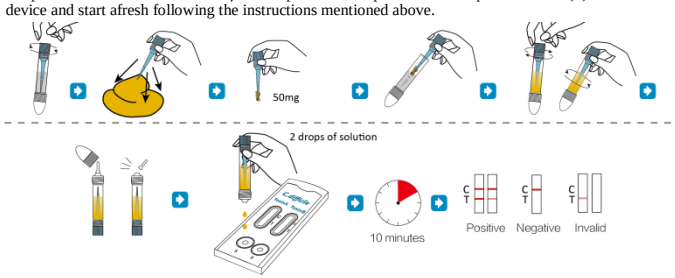
- Store the *C. difficile* Toxin A/B Test Kit at 2~30ºC when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

TEST PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Specimen collection and pre-treatment:
   1) Use clean, dry specimen containers for specimen collection. Best results will be obtained if the assay is performed within one hour after collection.
   Note: *If not tested within one hour, specimens collected in the specimen container may be stored for 1~2 days at 2~8ºC. For long-term storage, it is recommended to keep specimens below -20ºC.*
   2) **For solid specimens:** Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 3 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
   For liquid specimens: Hold the dropper vertically, aspirate fecal specimens, and then transfer 3 drops (approximately 100uL) of the liquid specimen into the sample diluent tube.
   3) Place the applicator back into the tube and screw the cap tightly. Be careful not to break the tip of the dilution tube.
   4) Shake the specimen collection tube to mix the specimen and the diluent buffer thoroughly.
2. Testing
   1) Remove the test device from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. To obtain a best result, the assay should be performed within one hour.
   2) Using a piece of tissue paper, break the tip of the dilution tube. Hold the tube vertically and dispense 2 drops of solution into the specimen well (S) of the test device.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
3. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the specimens contained in the sample diluents tube. Collect 100 µL of supernatant, dispense into the specimen well (S) of a new test device and start afresh following the instructions mentioned above.



RESULT INTERPRETATION

POSITIVE RESULT:

POSITIVE: One red band appears in the control region (C), and one red band in the test region (T). The shade of color may vary from pink to purple, but it indicates a positive result even with a faint line.

NEGATIVE RESULT:

NEGATIVE: Only one red band appears in the control region (C), and no band appears in the test region (T).

INVALID RESULT:

INVALID: No red band appears in the control region (C), whether a test band is present or not. Repeat invalid tests with a new sample, new test device and reagent. Insufficient sample volume, inaccurate operating procedure or expired tests may yield an invalid result. Contact your local distributor if the problem continues.

QUALITY CONTROL

- The *C. difficile* Toxin A/B Test Device has built-in (procedural) controls. Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the RED color located at the "C" line is present before reading the result.
- Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The *C. difficile* Toxin A/B Rapid Test Device is for professional in vitro diagnostic use, and should only be used for the qualitative detection of toxin A and/or B. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable toxogenic *C. difficile* bacteria are detectable with the *C. difficile* toxin A/B Rapid Test Device.
3. Toxin A and toxin B of *C. difficile* are very unstable, and may be degrades at room temperature. False-negative results may occur when specimens are not promptly tested or kept refrigerated until testing can be done.
4. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
5. Monoclonal antibodies may fail to detect, or detect with less sensitivity.
6. Failure to follow the Test Procedure and Result Interpretation may adversely affect test performance and/or invalidate the test result.
7. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
8. A high dose "hook effect" may occur where the color intensity of test band decreases as the concentration of antigen increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the test band.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Detection)

The limit of detection of *C. difficile* Toxin A/B Rapid Test is 5ng/mL for toxin A, and 3ng/mL for toxin B.

Clinical Sensitivity and Specificity

136 patient fecal samples were collected and tested on the *C. difficile* Toxin A/B Rapid Test and a commercial *C. difficile* Toxin A/B rapid test. Comparison for all subjects is shown in the following table:

	***C. difficile* Toxin A/B Rapid Test**		
Reference	**Positive**	**Negative**	**Total**
Positive	63	1	64
Negative	0	72	72
Total	63	73	136

Relative Sensitivity: 98.4%
Relative Specificity: 100%
Overall Agreement: 99.3%

Cross Reactivity

Cross reactivity with following organisms has been studied at 1.0 X 10^9 organisms/mL. The following organisms were found negative when tested with the *C. difficile* Toxin A/B Rapid Test (Feces).

Shigella spp	*Campylobacter* spp	*Escherichia coli*
Helicobacter pylori	*Enterococcus faecalis*	*Enterococcus faecium*
Norovirus	*Proteus mirabilis*	*Salmonella* spp
Staphylococcus aureus	*Rotavirus*	*Adenovirus*
Pseudomonas aeruginosa	*Neisseria meningitidis*	*Neisseria gonorrhea*
Group C Streptococcus	*Gardnerella vaginalis*	*Group B Streptococcus*
Klebsiella pneumoniae	*Candida albicans*	

LITERATURE REFERENCES

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2. Kuehne S. A., Cartman S. T., Heap J. T., Kelly M. L., Cockayne A., Mintn N. P. The role of toxin A and toxin B in *Clostridium difficile* infection. Nature in press Sep. 2010.
3. Michelle M. Riggs1, Ajay K. Sethi, Trina F. Zabarsky, Elizabeth C. Eckstein, Robin L. P. Jump1, and Curtis J. Donskey. Asymptomatic Carriers Are a Potential Source for Transmission of Epidemic and Nonepidemic *Clostridium difficile* Strains among Long-Term Care Facility Residents. Clin Infect Dis. (2007) 45 (8): 992-998.

GLOSSARY OF SYMBOLS

ρ	Catalog number	ϐ	Temperature limitation
ι	Consult instructions for use	Λ	Batch code
I	*In vitro* diagnostic medical device	ε	Use by
µ	Manufacturer	σ	Do not reuse

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Number: 1110018310
REV1.0/Effective date: 2016-11-23