Clostridium difficile Toxin A+Toxin B Combo Rapid Test Cassette (Feces)
Package Insert

REF ICGT-C62	English

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

【INTENDED USE】

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

【SUMMARY】

Clostridia (members of the genus Clostridium) are anaerobic, motile bacteria, ubiquitous in nature, and especially prevalent in soil. Under the microscope, they appear as long, irregular (often drumstick- or spindle-shaped) cells with a bulge at their terminal ends. Under Gram staining, *Clostridium difficile*(C. difficile)cells are Gram-positive and show optimum growth on blood agar at human body temperatures in the absence of oxygen. When stressed, the bacteria produce spores that are able to tolerate extreme conditions that the active bacteria cannot tolerate.
Clostridium difficile infection is associated with broad-spectrum antibiotic therapy and is the most common cause of infectious diarrhea in hospital patients. Pathogenic strains of C. difficile produce two protein exotoxins, toxin A and toxin B, which cause colonic mucosal injury and inflammation.*C. difficile* may become established in the human colon; [1,2,3]it is present in 2–5% of the adult population.

【PRINCIPLE】

Clostridium difficile Toxin A+ Toxin B Combo Cassette (Feces) is a qualitative, lateral flow immunoassay for the detection of Clostridium difficile in feces.
The *Clostridium difficile* Toxin A Rapid Test (Feces) is a qualitative, lateral flow immunoassay for the detection of Toxin A in feces. The membrane is precoated with antibody anti-Toxin A on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-Toxin A. The mixture migrates upward on the membrane chromatographically by capillary action to react with antibody anti-Toxin A 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.
The *Clostridium difficile* Toxin B Rapid Test (Feces) is a qualitative, lateral flow immunoassay for the detection of Toxin B in feces. The membrane is precoated with antibody anti-Toxin B on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-Toxin B. The mixture migrates upward on the membrane chromatographically by capillary action to react with antibody anti-Toxin B 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.

【REAGENTS】

The test cassette contains anti-Toxin A conjugated particles, anti-Toxin A coated on the membrane; anti-Toxin B conjugated particles, anti-Toxin B coated on the membrane.

【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】

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

【SPECIMEN COLLECTION AND PREPARATION】

The specimens must be tested as soon as possible after collection. If necessary, they 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.

【MATERIALS】

Materials Provided

- Test cassettes
- Package insert
- Specimen collection tubes with extraction buffer
- Dropper

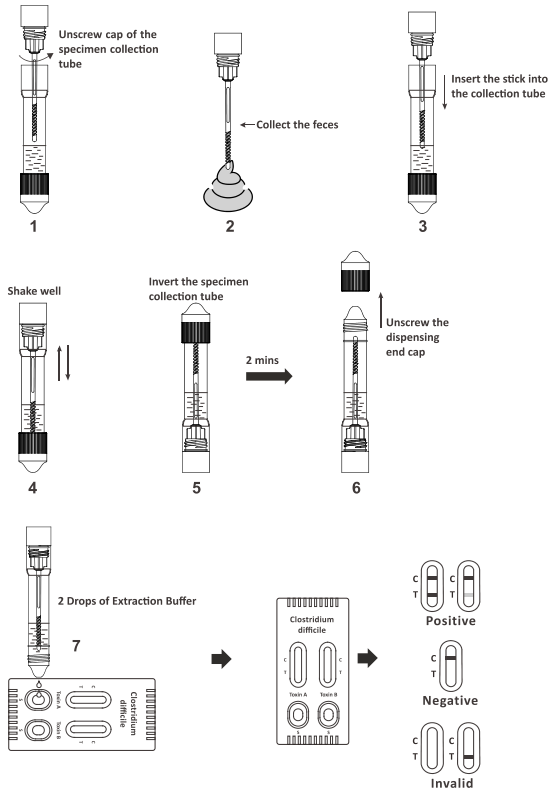
Materials Required But Not Provided

- Specimen collection containers
- Timer

【DIRECTIONS FOR USE】

Allow the test, specimen, buffer to reach 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 virus particles. 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:
   Unscrew the cap of the specimen collection tube , then randomly stab the specimen collection applicator into the fecal specimen in at least 3 different sites. Do not scoop the fecal specimen.
   Screw on and tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer.
3. Bring the pouch to room temperature before opening it. 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.
4. Hold the specimen collection tube upright and open the cap onto the specimen collection tube. Invert the specimen collection tube and transfer 2 full drops of the extracted specimen (approximately 80µL) 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.
5. Read results at 10minutes. Do not read results after 20 minutes.



【INTERPRETATION OF RESULTS】

POSITIVE: * Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the test region (T).
***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of Clostridium difficile, Toxin A and Toxin B present in the specimen. Therefore, any shade of line in the test region should be considered positive.
NEGATIVE: One color line appears in the control region (C). No apparent line appears in the test region (T).
INVALID: Control line 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.

【QUALITY CONTROL】

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

【LIMITATIONS】

1. The *Clostridium difficile* Toxin A+ Toxin B Combo Cassette (Feces) is for in vitro diagnostic use only. The test should be used for the detection of Clostridium difficile Toxin A and Toxin B in feces specimens only. Neither the quantitative value nor the rate of increase in Clostridium difficile Toxin A and Toxin B concentration can be determined by this qualitative test.
2. The *Clostridium difficile* Toxin A+ Toxin B Combo Cassette (Feces) will only indicate the presence of Clostridium difficile Toxin A and Toxin B in the specimen and should not be used as the sole criteria for Clostridium difficile Toxin A and Toxin B to be etiological agent for infectious diarrhea.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Clostridium difficile Infection.

【PERFORMANCE CHARACTERISTICS】

1)Sensitivity, Specificity and Accuracy
Clostridium difficile Toxin A +Toxin B Combo Rapid Test Cassette (Feces) has been compared with another leading commercial rapid test using clinical specimens
For Toxin A

Method		Other Rapid Test		
The *Clostridium difficile* Toxin A Rapid Test	Result	Positive	Negative	Total
	Positive	34	1	35
	Negative	0	48	48
Total Result		34	49	83

Relative sensitivity:34/34= 100.00% (95%CI: 91.57% ~ 99.92%)
Relative specificity:48/49= 97.96% (95%CI:89.14% ~ 99.94%)
Accuracy:(34+48)/(35+48) =98.80%(95%CI:93.47% ~ 99.97%)
*Confidence Intervals
For Toxin B

Method		Other Rapid Test		
The Clostridium difficile Toxin B Rapid Test	Result	Positive	Negative	Total
	Positive	52	0	52
	Negative	2	40	42
Total Result		54	40	94

Relative sensitivity:52/54= 96.30% (95%CI: 87.25% ~ 99.54%)
Relative specificity:40/40=100.00% (95%CI:92.78% ~ 100%)
Accuracy:(52+40)/(52+2+40) =97.87%(95%CI:92.52% ~ 99.74%)
*Confidence Intervals

2) Precision
To check intra-batch accuracy, 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, some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

3) Cross Reaction
Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: Campylobacter coli, Campylobacter jejuni, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Escherichia hermanii,Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Legionella bozemanii (sg1), Legionella lonbeachae, Legionella pneumophila (sg1), Moraxella catarrhalis, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium tuberculosis, Mycoplasma hominis, Neisseria meningitides (sg B & C), Neisseria sicca, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus (Gr B, C, F, G), Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahemolyticus, Yersinia enterocolitica (type1, 3, 9).

【BIBLIOGRAPHY】

1. Ciarán P. Kelly, MD and J. Thomas LaMont, MD，Clostridium difficile Infection, Annual Review of Medicine ,49,1998
2. Ramadass Balamurugan, V. Balaji and Balakrishnan S. Ramakrishna: Estimation of faecal carriage of *Clostridium difficile* in patients with ulcerative colitis using real time polymerase chain reaction, Indian Journal of Medical Research, p.472-477, May 2008
3. E. J. Kuijper, B. Coignard and P. Tüll: Emergence of *Clostridium difficile*-associated disease in North America and Europe, Review Clinical Mocrobiology and Infections, 12 suppl 6, p. 2-18, Oct. 2006
4. Ramsey L. et al: Fulminant *Clostridium difficile*: an underappreciated and increasing cause of death and complications, Annals of Surgery 235 (3) p. 363-372: Mar. 2002
5. Wren MW., Kinson R., Sivapalan M., Shemko M., Shetty NR.: Detection of *Clostridium difficile* infection: a suggested laboratory diagnostic algorithm, British Journal of Biomedical Sciences, 66(4) p. 175-179, 2009.

6. Shetty N., Wren MW., Coen PG.: The role of glutamete dehydrogenase for the detection of *Clostridium difficile* in faecal samples: a meta-analysis, Journal of Hospital Infections, 77(1), p.1-6,Jan.2001.

Index of Symbols

	Consult Instruction for use		Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	**REF**	Catalog #
	Do not use if package is damaged				

Manufacturer

Hangzhou Biotest Biotech Co., Ltd.
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Yuhang District, Hangzhou, P. R. China

CE

EC REP

Shanghai International
Holding Corp. GmbH (Europe)
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