todylaboratories®

Rapid Tests

CE IVD REF C62RT1014

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

A rapid test for the qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase(GDH), Toxin A and Toxin B in feces.
For professional *in vitro* diagnostic use only.

INTENDED USE

The *Clostridium difficile* GDH+Toxin A+B Combo Rapid Test Cassette (Feces)is a rapid chromatographic immunoassay for the qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase(GDH), 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

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

Material Provided

• Test cassettes • Specimen collection tubes with extraction buffer • Package insert • 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 maximum 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:

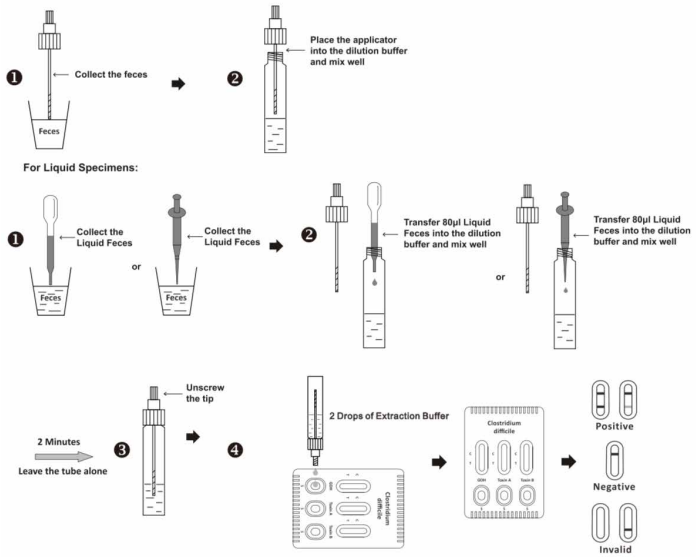
• For Solid Specimens:

Unscrew the cap of the specimen collection tube，then randomly stab the specimen collection applicator into the fecal specimen in 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 or Pipette vertically, aspirate fecal specimens, and then transfer 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 tube alone for 2 minutes.

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* GDH, 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.

LIMITATIONS

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.

PERFORMANCE CHARACTERISTICS

1) Sensitivity - Specificity

1. *Clostridium difficile* GDH+Toxin A+B Combo Rapid Test Cassette (Feces) has been compared with another leading commercial rapid test using clinical specimens

For *Clostridium Difficile* GDH:

Method		Other Rapid Test		
Clostridium difficile GDH Rapid Test	Result	Positive	Negative	Total
	Positive	91	1	92
	Negative	0	226	226
Total Result		91	227	318

Relative sensitivity:91/91>99.99% (95%CI: 96.76% ~ 100.00%)
Relative specificity:226/227= 99.56% (95%CI:97.57% ~ 99.99%)
Accuracy:(91+226)/(91+226+1) =99.69% (95%CI:98.26% ~ 99.99%)
*Confidence Intervals

For Toxin A:

Method		Other Rapid Test		
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>99.99% (95%CI: 91.57% ~ 99.92%)
Relative specificity:48/49= 97.96% (95%CI:89.14% ~ 99.94%)
Accuracy:(34+48)/(35+48+1) =98.80%(95%CI:93.47% ~ 99.97%)
*Confidence Intervals

For Toxin B:

Method		Other Rapid Test		
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>99.99% (95%CI:92.78% ~ 100%)
Accuracy:(52+40)/(52+2+40) =97.87%(95%CI:92.52% ~ 99.74%)
*Confidence Intervals

2) Accuracy

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).

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INDEX OF SYMBOLS

	Manufacturer
IVD	For *in vitro* diagnostic use only
LOT	Lot number
REF	Product code
	Use by
2°C 30°C	Store between 2-30°C
CE	European Conformity
	Consult Instructions For Use
Σ	Tests per kit
	Do not use if package is damaged
	Do not reuse

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