PathFlow™ C.difficile GDH and Tox A/B Combi (EN)

MICROGEN BIOPRODUCTS

Instructions for Use

INTENDED USE

The PathFlow™ C.difficile GDH and Tox A/B Combi Cassette is a rapid chromatographic immunoassay for the qualitative detection of Clostridium difficile GDH, Toxin A and Toxin B in the human faeces 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-diarrhoea 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 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]

PRINCIPLE

The PathFlow™ C.difficile GDH and Tox A/B Combi Cassette detects three distinct antigens in faecal specimens for *C. difficile*, *viz.*, GDH, Toxin A and Toxin B on three different test strips in a single test cassette, thus simultaneously detecting three antigens specific of *Clostridium difficile*.

For *C. difficile*-specific GDH Testing

The membrane is precoated with anti-*C.diff.* GDH antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-*C.diff* GDH antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.diff GDH antibody on the membrane and generate a coloured line.

For *C. difficile*-specific Toxin A Testing

The membrane is precoated with 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 coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result.

For *C. difficile*-specific Toxin B Testing

The membrane is precoated with 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 coloured line. The presence of this coloured line in the test line region indicates a positive result, while its absence indicates a negative result.

To serve as a procedural control, a coloured line will always appear in the control line region of all the three test strips, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test cassette contains anti-Clostridium difficile GDH, anti-Clostridium difficile Toxin A and anti-Clostridium difficile Toxin B particles gold conjugate pair with anti-Clostridium difficile GDH, anti-Clostridium difficile Toxin A and anti-Clostridium difficile Toxin B coated on the membrane.

MATERIALS

Provided: Test cassettes, Package Insert, Specimen collection tube with buffer, Dropper

Not Provided: Specimen collection / stool container, timer.

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

SPECIMENS

The stool specimens must be tested as soon as possible after collection. If necessary, original faeces 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.

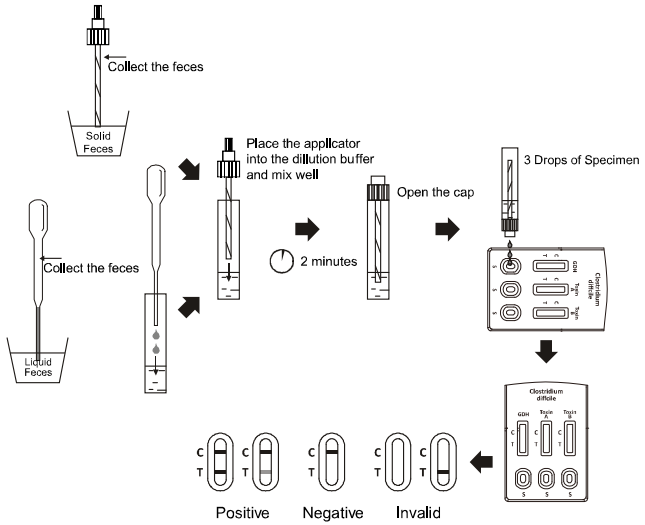
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.

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 collect faecal specimens: Collect sufficient quantity of faeces (1-2mL or 1-2g) 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 faecal specimens:
   For Solid Specimens: Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the faecal specimen at least 3 different sites to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). Do not scoop the faecal specimen.
   For Liquid Specimens: Hold the dropper vertically, aspirate faecal 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.
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 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 specimen well of the test cassette, 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 120µL 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

(Please refer to the illustration above)

The test results appear in three different test windows respectively for GDH, 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 distinct coloured lines appear.** One coloured line should be in the control line region (C) and another apparent coloured line should be in the test line region (T).

***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentration of *Clostridium difficile* antigens present in the specimen. Therefore, any shade of colour in the test line region (T) should be considered positive.

NEGATIVE: One coloured 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 strip immediately and contact your local distributor.

QUALITY CONTROL

An internal procedural control is included in the test. A coloured 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 PathFlow™ C.difficile GDH and Tox A/B Combi Cassette 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.

EXPECT VALUE

In a healthy individual's faecal specimens, *Clostridium difficile* test should give negative test result for any of the antigens tested.

The PathFlow™ C.difficile GDH and Tox A/B Combi Cassette has been compared with another leading commercial rapid test.

The correlation between two system is 98.5% for *C.diff* GDH and 98.5% for *C.diff* Toxin A+Toxin B.

DETECTION LIMIT

Detection limit values of PathFlow™ C.difficile GDH and Tox A/B Combi Cassette was 1ng/ml for GDH, 2ng/ml for Toxin A and 1ng/ml for Toxin B.

SENSITIVITY - SPECIFICITY

Clostridium difficile GDH Results

Method	Results	Other Rapid Test		Total Results
		Positive	Negative	
PathFlow™ C.difficile GDH and Tox A/B Combi Cassette	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

***Clostridium difficile* Toxin A +Toxin B Results**

Method	Results	Other Rapid Test		Total Results
		Positive	Negative	
PathFlow™ C.difficile GDH and Tox A/B Combi Cassette	Positive	56	2	58
	Negative	1	141	142
Total Results		**57**	**143**	**200**

Relative Sensitivity: 98.2% (95%CI:*90.6%-99.9%)
Relative Specificity: 98.6% (95%CI:*95.0%-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 PathFlow™ C.difficile GDH and Tox A/B Combi Cassette.

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

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TABLE OF SYMBOLS

SYMBOL	DEFINITION
LOT	Batch Code/Lot Number
IVD	In Vitro Diagnostic Medical Device
REF	Catalogue Reference
	Upper and Lower Temperature Limit
	Expiry Date
	Manufacturer and Address Details
	Date of Manufacture
	Please consult Instructions for Use
	Sufficient for
	Do not use if package is damaged
CE	CE Mark
	Caution

This document applies to the following product codes:
M588CE / M588a / M588b

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