Proflow™ *C. difficile* GDH

(for *In Vitro* Diagnostic use only)

PRODUCT CODE PL.3001

20 Tests

INTENDED USE

The Proflow™ *C. difficile* GDH is a qualitative lateral flow test for the detection of *Clostridium difficile* glutamate dehydrogenase (GDH) in faecal specimens. The Proflow™ *C. difficile* GDH is intended for use as an aid in the diagnosis of *C. difficile* infections. The test detects GDH and will not differentiate between toxigenic and non- toxigenic strains of *C. difficile*. Like alternative *C. difficile* tests, results should be considered in conjunction with patient history and additional laboratory investigations. For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

Clostridium difficile is an anaerobic Gram-positive spore-forming bacillus. The key feature in enabling it to persist in patients and the physical environment for long periods and thereby facilitating its transmission is the ability of *C. difficile* to form spores. *C. difficile* is transmitted via the faecal-oral route.

C. difficile is the principal pathogen related to antibiotic associated diarrhoea and/or pseudomembranous colitis in hospitalised patients.

Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonisation. However, if the normal colonic flora is altered, resistance to colonisation is lost. Thus, any factor associated with alteration of the normal enteric flora increases the risk of *C. difficile* colonisation after exposure to antibiotics, especially those with broad- spectrum activity such as penicillins, cephalosporins and clindamycin.

C. difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for clinical manifestations, which range from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon, and death.

PRINCIPLE OF THE TEST

The Proflow™ *C. difficile* GDH test is a single use rapid membrane immunoassay for the qualitative detection of *C. difficile* GDH antigen in human faeces.

Monoclonal antibodies to *C. difficile* GDH antigen are coated onto the test line region of the strip. During testing the sample migrates along the membrane by capillary action and is allowed to react with the conjugate on the test strip. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate.

A green line should always appear in the control line region to show that sufficient volume was added, proper flow was obtained and the reagents functioned correctly. Failure of the control line to appear, whether or not test lines are present, indicates an invalid assay. The test is interpreted by the presence or absence of visibly detectable coloured lines in the test region at 10 minutes or less depending on the concentration of antigen present.

A positive result will show a pink/red test line and a green control line, indicating that *C. difficile* GDH antigen is present in the sample. A negative test result, read at 10 minutes, will show only a green control line, indicating that *C. difficile* GDH antigen was not detectable in the sample.

MATERIALS PROVIDED

- PL.3101 Proflow™ *C. difficile* GDH Test Device: 20 Devices
- PL.3102 Proflow™ *C. difficile* GDH Sample Diluent
- PL.3103 Proflow™ Positive Control
- Plastic Pipette: 40
- Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Sample collection container
- Timer or stopwatch
- Biohazard disposal container
- Disposable gloves

STABILITY AND STORAGE

- Store all components at 2-30°C.
- Do not freeze or overheat.
- Do not use beyond the expiration date printed on the outer package label.
- The test kits should be kept away from direct sunlight, moisture and heat.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Tests are for single use only. Do not reuse.
- Reagents are provided at the necessary working strength. Do not dilute reagents.
- Do not interchange reagents between kits with different lot numbers.
- Do not use kits or reagents beyond the stated expiration dates.
- Microbial contamination of reagents may decrease the accuracy of the assay.
- Treat all materials as if they were infectious and dispose of all material in accordance with local regulation.
- The test must be carried out within 2 hours of opening the sealed bag.

SAMPLE STORAGE AND COLLECTION

- Collect sufficient quantity of faeces (1-2g or mL for liquid samples)
- Faecal samples should be collected in clean and dry containers (no preservatives or transport media).
- Samples may be stored at 2-8°C for 1-2 days or at -20°C for longer periods before testing.
- Allow all samples to equilibrate to room temperature before testing.
- Freezing and thawing cycles are not recommended.

TEST PROCEDURE

Allow the tests, faeces samples and reagents to reach room temperature prior to testing.

1. Add 1 ml of Sample Diluent to a tube.
2. Transfer a pea-sized piece of faecal sample, approx. 50 mg, to the tube of Sample Diluent. For liquid faeces, aspirate the sample with a dropper and add 50 µL into the tube of Sample Diluent.
3. Mix to ensure good sample dispersion.
4. Let the sample settle for 2 minutes to allow any large particles to collect at the bottom of the tube. Alternatively, spin down for 30 seconds in a centrifuge.
5. Remove the Proflow™ *C. difficile* GDH test from its sealed pouch.
6. Dispense 3 drops (100 uL) into the sample well on the test (S).
7. Read the result at 10 minutes.

Procedure for Positive Control:

1. Open the pouch, take out the cassette and place it on a clean flat surface.
2. Dispense 3 drops (100 µl) of Positive Control into the circular window.
3. Read the result after 10 minutes.

QUALITY CONTROL PROCEDURE

It is recommended that a positive and negative control be used to ensure that the test is performing as expected.

The control line (C) is a procedural control and will show that the test has been performed correctly; proper flow occurred and that the test reagents functioned as expected. When a green line appears at the control line position this indicates the test has been performed correctly. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the Interpretation of Results section). A positive control is provided in the kit.

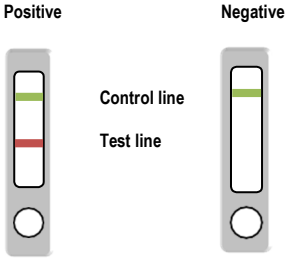
INTERPRETATION OF RESULTS

Positive

A pink/red line of any intensity appears in the test window at the test line position; a green line will appear at the control line position. This indicates a reactive result that is interpreted as positive for *C. difficile* GDH.

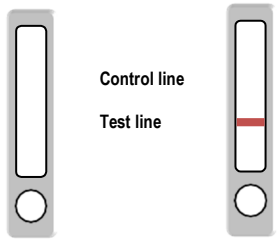
Negative

A single green line of any intensity appears in the test window at the control line position. There is no line at the test line position. This indicates a non-reactive result that is interpreted as negative for *C. difficile* GDH.



Invalid

No line appears in the test window at the control line position. This is an invalid result and cannot be interpreted. This is irrespective of whether or not a pink/red line appears in the test window at the test line position. If either condition below occurs, the test should be repeated with a new test.



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LIMITATIONS OF THE PROCEDURE

- Proflow™ *C. difficile* GDH test will only indicate the presence of *C. difficile* GDH in the sample (qualitative detection) and should be used for the detection of *C. difficile* GDH in faeces samples only. Neither the quantitative value nor the rate of increase in *C. difficile* GDH concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some faeces samples can decrease the intensity of the control line.
- The test must be carried out within 2 hours of opening the sealed bag.
- Avoid Antimicrobials, Proton pump inhibitors and Bismuth for 10 days prior to testing.
- Mucous and/or bloody faecal samples can cause non-specific reactions. A positive result with a mucous or bloody sample should be confirmed with other techniques.
- 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 *C. difficile* GDH infection.
- This test provides a presumptive diagnosis of *C. difficile* GDH infections. All results must be interpreted together with other clinical information and laboratory findings available to the clinician.

PERFORMANCE CHARACTERISTICS

EXPECTED VALUES

C. difficile is associated with 95-100% of cases of pseudomembranous colitis, 60-75% of cases of antibiotic-associated colitis, and 35% of cases of antibiotic-associated diarrhoea cases.

LIMIT OF DETECTION

The detection limit range for the *Clostridium difficile* GDH Device is 0.78-0.10 ng/ml of *C. difficile* GDH recombinant protein.

SENSITIVITY AND SPECIFICITY

An evaluation was performed against another commercial lateral flow assay. Results were confirmed by a qPCR technique (VAISURE *Clostridium difficile* Real Time Detection Kit, Certest Biotec), and are as follows:

		Competitor Kit		
		Positive	**Negative**	**Total**
IC test: Proflow™ *C.difficile* GDH	**Positive**	51	0	51
	Negative	0	75	75
	Total	51	75	126

		***C. difficile* Ag (GDH) Device vs. competitor kit**
		95% CI (Confidence Interval)
Sensitivity	100.0%	93.0-100.0%
Specificity	100.0%	95.2-100.0%
PPV	100.0%	93.0-100.0%
NPV	100.0%	95.2-100.0%

CROSS-REACTIVITY

An evaluation was performed to determine the cross-reactivity of the Proflow™ GDH test. There was no cross-reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces:

- Adenovirus
- Astrovirus
- Calprotectin (human)
- *Campylobacter coli / jejuni*
- *Clostridium difficile* Toxin A / B
- *Clostridium perfringens*
- *Entamoeba histolytica*
- *Escherichia coli* O:111 / O:026 / O157:H7
- Giardia
- *Helicobacter pylori*
- Haemoglobin (human / bovine / pig)
- Lactoferrin (human / bovine)
- Legionella
- *Listeria monocytogenes*
- Norovirus GI / GII
- Rotavirus
- *Salmonella enteritidis / paratyphi A/ typhi / typhimurium*
- *Shigella boydii / dysenteriae / flexneri / sonnei*
- *Streptococcus pneumococcal / pyogenes*
- Transferrin (human / bovine)
- *Yersinia enterocolitica* O:3 / O:9

REFERENCES

- Wren, M.W.D, et al. Laboratory diagnosis of *Clostridium difficile* infection. An evaluation of tests for faecal toxin, glutamate dehydrogenase, lactoferrin and toxigenic culture in the diagnostic laboratory. *British Journal of Biomedical Science.* 66 (1), 2009.
- Vaishnavi, Ch. Clinical spectrum & pathogenesis of *Clostridium difficile* associated diseases. *Indican J. Med. Res.* 131, April 2010, pp. 487-499.
- Poutanen, S. M. et al. *Clostridium difficile*-associated diarrhoea in adults. *CMAJ.* 171 (1), July 2004, pp. 51-58.

= Use by

LOT = Lot number

REF = Catalogue number

= Manufacturer

EC REP = Authorized Representative in the European Community

= Contains sufficient for <n> tests

IVD = In vitro diagnostic medical device

= Temperature limitation

= Consult instructions for use

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