Proflow™ *C. difficile* Toxin A/B

(for *In Vitro* Diagnostic use only)

PRODUCT CODE PL.3002 **20 Tests**

INTENDED USE

The Proflow™ *C. difficile* Toxin A/B test is a rapid chromatographic immunoassay for the simultaneous qualitative detection of *C. difficile* Toxin A-B antigens in human faecal specimens to aid in the diagnosis of *C. difficile* infection. For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

Clostridium difficile is an anaerobic, Gram-positive, spore-forming bacillus. Although themajority of the strains isolated are non-toxigenic, some of them can release two high-molecular-weight toxins, toxin A and toxin B. Toxin A is an enterotoxin and Toxin B a cytotoxin. These toxins can cause watery diarrhoea and may cause pseudomembranous colitis (PMC) in the presence of broad-spectrum antibiotics and other agents. Disease incidence increases with age, a compromised immune system, and the duration of hospital stay.

C. *difficile* produces acid resistant spores and can be transmitted by contaminated surfaces or physical contact. *C. difficile* is the most commonly identified cause of nosocomial diarrhoea in adults.

C. difficile infections (CDI) are classified into two groups according to their severity: post antibiotic diarrhoea and PMC. PMC represents 7-9% of CDI's. PMC usually begins with watery diarrhoea accompanied by fever and abdominal pain. Pseudomembranous lesions may be visible on endoscopic examination. Mortality due to *C. difficile* infections varies from 0.6 to 1.5%, but can reach as high as 35 to 50% in susceptible populations.

C. difficile infection can be diagnosed by the detection of the toxins or by the detection of glutamate dehydrogenase (GDH) directly in stool samples. All isolates of *C. difficile* produce GDH so GDH testing can be used as a screening method for the detection of *C. difficile*. Subsequent testing for toxin production is required to confirm diagnosis.

PRINCIPLE OF THE TEST

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

Monoclonal antibodies to *C. difficile* Toxin A/B antigens are coated onto the test line regions 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* Toxin A or B 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* Toxin A/B antigen was not detectable in the sample.

MATERIALS PROVIDED

- PL.3201 Proflow™ *C. difficile* Toxin A/B Test Devices: 20 devices
- PL.3202 Proflow™ *C. difficile* Toxin A/B Sample Diluent
- 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 as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable to the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

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

Use a separate sample collection vial and test for each sample or control. Allow the tests, faeces samples and buffer 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* Toxin A/B 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.

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).

INTERPRETATION OF RESULTS

Positive

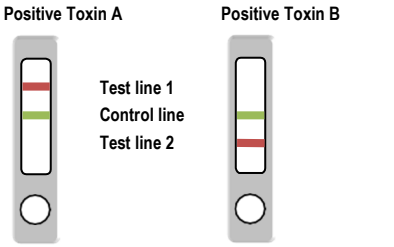
Toxin A positive: Two lines appear across the central window; a red line at the test line 1 position, and a green control line.

Toxin B positive: Two lines appear across the central window; a red line at the test line 2 position, and a green control line.

Toxin A-B positive: Three lines appear across the central window, the two red test lines and the green control line.

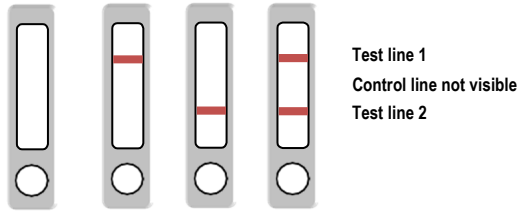
Negative

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



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 pink/red lines appear in the test window at the test line positions. 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* Toxin A/B test will only indicate the presence of Toxins A and B of *C. difficile* in the sample (qualitative detection) and should be used for the detection of *C. difficile* Toxin A/B in faeces samples only. Neither the quantitative value nor the rate of increase in *C. difficile* Toxin A/B 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* infection.
- This test provides a presumptive diagnosis of *C. difficile* infection. All results must be interpreted together with other clinical information and laboratory findings available to the clinician.

PERFORMANCE CHARACTERISTICS

EXPECTED VALUES

Clostridium 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* device is 62.5-7.8 ng/ml of Toxin A.
The detection limit range for the *Clostridium difficile* device is 6.24-0.39 ng/ml of Toxin B.

SENSITIVITY AND SPECIFICITY

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

		Competitor Kit		
		Positive	**Negative**	**Total**
IC test: Proflow™ *C.difficile* Toxin A-B (Toxin A)	**Positive**	25	0	25
	Negative	2	99	101
	Total	27	99	126

		***C. difficile* Toxin A-B (Toxin A) Device vs. competitor kit**
		95% CI (Confidence Interval)
Sensitivity	92.6%	75.7-99.1%
Specificity	100.0%	96.3-100.0%
PPV	100.0%	86.3-100.0%
NPV	98.0%	93.0-99.8%

		Competitor Kit		
		Positive	**Negative**	**Total**
IC test: Proflow™ *C.difficile* Toxin A-B (Toxin B)	**Positive**	32	0	32
	Negative	2	92	94
	Total	34	92	126

		***C. difficile* Toxin A-B (Toxin B) Device vs. VIASURE kit**
		95% CI (Confidence Interval)
Sensitivity	94.1%	80.3-99.3%
Specificity	100.0%	96.1-100.0%
PPV	100.0%	89.1-100.0%
NPV	97.9%	92.5-99.7%

CROSS REACTIVITY

An evaluation to determine the cross reactivity of *C. difficile* Toxin A-B was performed. There was no cross reactivity with common gastrointestinal pathogens occasionally present in faeces:

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