PRO-LAB DIAGNOSTICS

Proflow™ *C. difficile* GDH

(for *in vitro* diagnostic use)

PRODUCT CODE: PL.3001C

Σ 20

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. This test is intended for laboratory use only.

SUMMARY AND EXPLANATION

Clostridium difficile is an anaerobic, gram-positive, spore-forming bacillus. Although the majority of the strains isolated are non-toxigenic, some of them will produce toxins A or 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

Proflow™ *C. difficile* GDH is a qualitative, lateral flow immunoassay for the detection of GDH antigen in stool. The assay uses antibodies specific to GDH coated onto the membrane in the test line. During testing, the GDH present in the stool specimen reacts with the anti-GDH antibody (conjugated with gold particles) and migrates up the membrane by capillary action. This in turn reacts with the anti-GDH antibodies coated in the test line. The presence of a coloured line test indicates a positive result, while its absence indicates a negative result. A second capture line comprising rabbit anti-mouse antibodies serves as a procedural control and must be observed in a valid test.

MATERIALS PROVIDED

- Test Cassette (PL.3101): 20 cassettes packaged with a desiccant in individual aluminum pouches.
- Sample Diluent (PL.3102): 1 dropper bottle of Sample Diluent containing 21 ml of buffer containing proteins and preservative.
- Positive Control (PL.3103): 1 dropper bottle of ready to use Positive Control containing 0.5 ml of recombinant GDH in a buffer with proteins and preservative.
- Disposable pipettes: 20 disposable pipettes for the collection of liquid samples.
- Instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Test Tubes for sample preparation
- Pipette
- Vortex

STABILITY AND STORAGE

- Reagents should be stored between 2-30°C until the expiry date indicated on the label.
- Do not open until ready to use as the test is sensitive to humidity and to heat.
- **Do not freeze.**
- Do not use the kit beyond the expiration date.
- The dropper bottles must be closed after each use and stored with the other components in the box. After first opening, the buffer is stable until the expiry date indicated on the bottle.

PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only.
2. Follow the instructions for use carefully.
3. Test cassettes and plastic pipettes provided in the kit are intended for single use only. Do not re-use.
4. Do not interchange or mix reagents from different kits and lots.
5. Do not use a test cassette if the aluminum pouch has been opened or damaged.
6. Universal precautions should be taken in handling, processing and discarding all materials used to perform the test.
7. This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease
8. The addition of preservatives such as SAF (Solution of sodium acetate, acetic acid and formalin 10%) can cause false negative results.

SPECIMEN COLLECTION AND STORAGE

1. Testing should be performed as soon as possible after the specimen has been collected.
2. Samples can be stored at 2-8°C for up to 48 hours or frozen at -20°C for one month. For long term storage specimens must be frozen at -80°C.
3. If the specimen has been refrigerated allow it to come to room temperature before testing.
4. Frozen samples must be completely thawed and mixed prior to testing.
5. Specimens should not be frozen and thawed repeatedly.
6. Specimens can be stored in Carey Blair Transportation Medium up to 1 week at 4°C without impacting test performance.
7. When testing faecal samples with Cary Blair Transportation Medium, mix 300 µl faecal sample with 300 µl Dilution Buffer, add 100 µl of the dilution to the sample well of the cassette and read the result after 15 minutes.

TEST PROCEDURE

1. The specimen, test cassettes and reagents should be brought to room temperature before testing.
2. Open the aluminum pouch, take out the cassette and place it on a clean and flat surface.
3. Add 1 ml of Sample Diluent to a tube.
4. Using one of the disposable pipettes, transfer 100 µl of liquid stool (up to the first mark on the pipette) to the tube of Sample Diluent. If the specimen is formed, transfer a pea sized piece (approximately 100 mg) to the sample tube.
5. Mix with a vortex. (Dilution to be used within one hour)
6. Take 100 µl of the dilution and add it in the sample well (S) on the test cassette.
7. Read the result after 15 minutes.

Procedure for Positive Control:

1. Open the aluminum pouch, take out the cassette and place it on a clean and flat surface.
2. Dispense 2 drops (100 µl) of Positive Control in the sample well (S) of the cassette.
3. Read the result after 15 minutes.

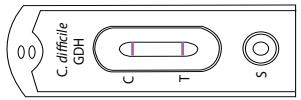
QUALITY CONTROL PROCEDURE

A Positive Control is supplied with the kit in order to validate the device.
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.
A brownish stain can appear on the membrane. This will have no influence on the reading of the test.

INTERPRETATION OF RESULTS

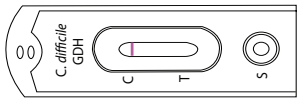
POSITIVE:

A positive result is indicated by the presence of two distinct purple bands. One in the control line (C) and one in the test line (T). Any line in these areas despite the intensity of the bands should be read as positive.



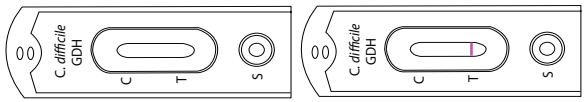
NEGATIVE:

Only one purple band appears at the control line (C). There must be no band at the test line (T).



INVALID:

If no lines appear on the test cassette or a line appears on the test line only the result is invalid. If this happens, review the procedure and repeat the test with a new test cassette and a new sample tube. If the problem persists, contact Pro-Lab immediately.



LIMITATION OF THE PROCEDURE

1. This test is based on the detection of the presence of the GDH protein of *C. difficile* in stool. The test should not be used in isolation to diagnose *C. difficile* associated disease.
2. GDH is a characteristic enzyme produced by *C. difficile* but the test does not distinguish between toxigenic and non-toxigenic strains. Other tests are required to confirm the presence of toxigenic strains of *C. difficile*.

Canada ☎ 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit #4, Richmond Hill, ON, L4B 1K3

U.S.A. ☎ 800 522 7740 Fax 800 332 0450
21 Cypress Blvd., Suite 1070, Round Rock, Texas, 78665-1034

U.K. ☎ 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside CH62 3QL

PERFORMANCE CHARACTERISTICS

Clinical Study

Clinical performance of Proflow™ *C. difficile* GDH was evaluated at two external clinical trial sites (Table 1).

Table 1: Sensitivity, Specificity, and Percent Agreement Values of Proflow™ *C. difficile* GDH after Resolution of False Positives

Location	Culture Method	True Positive	False Negative	True Negative	False Positive	Sensitivity	Specificity
Site 1*	Bacterial culture	80	8	366	26	90.9% (82.4-95.7) ***	93.4% (90.3-95.5)
Site 2*	Toxigenic culture	21	3	266	14	87.5% (66.5-96.7)	95.5% (91.6-97.1)
Combined	----	101	11	632	40	90.2% (82.7-94.8)	94.0% (91.9-95.7)

* Samples that were initially culture negative and Proflow positive were tested using a cleared nucleic acid amplification test for the detection of toxin A and B genes.

** Samples that were initially culture negative and Proflow positive were retested after culture enrichment.

***95% confidence intervals

Transport Media

Culture positive (n=13) and negative samples (n=9) were tested after storage for 1 week in Cary Blair Transportation Medium at 4°C. All samples produced the expected results after storage in Cary Blair Transportation Medium for 1 week. Storage in Cary Blair Transportation Medium does not impact test performance.

Detection Limit of the Test

The Proflow™ *C. difficile* GDH limit of detection was between 2.65 x 10^5 and 1.49 x 10^6 colony forming units per ml with two *C. difficile* strains.

Cross Reactivity

A total of 46 microorganisms were tested for cross-reactivity by testing in culture media or after adding them to *C. difficile* negative stool samples. Only one organism, *Clostridium sporogenes*, was reactive when tested in stool samples. This organism is not typically found in stool samples and is thus unlikely to cause false positive results.

Interfering Substances

Potential interfering substances were tested by adding them to stool samples spiked with *C. difficile*. The substances testing included antacids, antidiarrheals, laxatives, antibiotics, faecal fat, blood, mucus, contrast media, and antiflatulence medication. None of the tested substances interfered with the test or produced false positive results.

REFERENCES

1. **Fenner L., Widmer A.F., Goy G., Rudin S., Frei R.** (2008). Rapid and Reliable Diagnostic Algorithm for Detection of *Clostridium difficile*. J. Clin. Microbiol. 46 (1). 328-330.
2. **Barbut F., and JC. Petit.** (2001). Epidemiology of *Clostridium difficile*-Associated Infections. Clin Microbiol Infect. 7 (8). 405-410
3. **Shea-idsa guideline. Cohen S.H.; Gerding D.N., Johnson S., Kelly C.P., Loo V.G., McDonald L.C., Pepin J., Wilcox M.H.** (2010). Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) Infection control and hospital epidemiology. 31 (5)
4. **Eastwood K., Else P., Charlett A., Wilcox M.** (2009). Comparison of Nine Commercially Available *Clostridium difficile* Toxin Detection Assays, a Real-Time PCR Assay for *C. difficile* tcdB, and a Glutamate Dehydrogenase Detection Assay to Cytotoxin Testing and Cytotoxigenic Culture Methods. J. Clin. Microbiol. 47(10). 3211-3217.
5. **Crobach M.J.T, Dekkers O.M., Wilcox M.H., Kuijper** E.J. European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2009). Data Review and Recommendations for Diagnosing *Clostridium difficile*-infection (CDI). Clin Microbiol Infect. 15. 1053–1066.