Proflow™ *C. difficile* GDH

(for *In Vitro* Diagnostic use only)

PRODUCT CODE PL.3001 Σ **20**

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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 EXPLINATION

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.
- Proflow™ Pastettes: 40 disposable pastettes
- Instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Test Tubes for sample preparation
- 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

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

SAMPLE STORAGE AND COLLECTION

- Testing should be performed as soon as possible after the specimen has been collected.
- 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.
- If the specimen has been refrigerated allow it to come to room temperature before testing.
- Frozen samples must be completely thawed and mixed prior to testing.
- Specimens should not be frozen and thawed repeatedly
- Specimens can be stored in Carey Blair Transportation Medium up to 1 week at 4°C without impacting test performance.
- 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 pouch, take out the cassette and place it on a clean 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 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 for ten seconds. (Dilution to be used within one hour).
6. 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.
7. Take 100 µl of the dilution (avoiding the particulates at the bottom of the tube) and add it in the sample well (S) on the test cassette.
8. Read the result after 15 minutes.

Procedure for Positive Control:

1. Open the pouch, take out the cassette and place it on a clean 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.

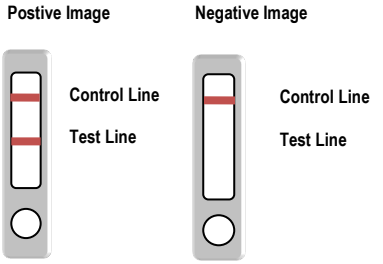
INTERPRETATION OF RESULTS

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.

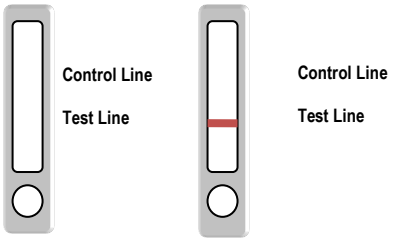
Negative

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.



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LIMITATIONS 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*.

PERFORMANCE CHARACTERISTICS

Clinical Study

A hospital evaluation was performed comparatively to toxigenic culture, results are presented in the following table.

		Toxigenic Culture		
		Positive	Negative	Total
Proflow™ *C. difficile* GDH	Positive	23	14	37
	Negative	1	266	267
	Total	24	280	304

Sensitivity: 95.8% [76.9% - 99.8%]*
Specificity: 95.0% [91.6% - 97.1%]*
Positive Predictive Value: 62.2% [44.8% - 77.1%]*
Negative Predictive Value: 99.6 % [97.6% - 100%]*
* 95% confidence intervals

Detection Limit of the Test

Studies have shown that the test has a detection limit of 1.58 ng/ml with a recombinant protein in sample diluent. This corresponds to 17 ng/mg in a stool specimen.

Interference

Cross reactivity was evaluated by assaying samples with the addition of blood (20%), Loperamide (4%) and SMECTA® (5%). No interference was obtained with any of the agents.

REFERENCES

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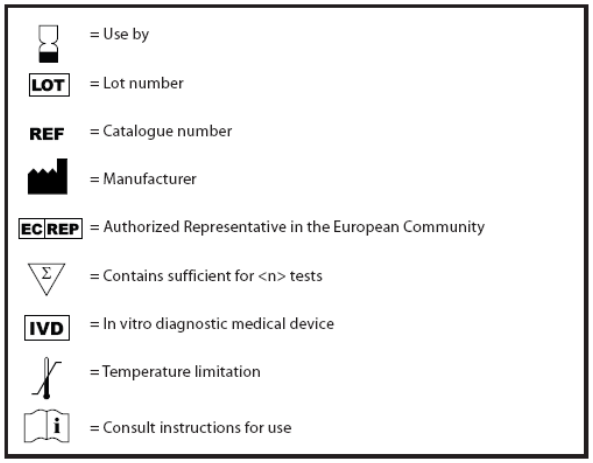
5. Crobach M.J.T, Dekkers O.M., Wilcox M.H., Kuijper E.J. European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2009). Data Review and Recommendations for Diagnosing *Clostridium difficile*-infection (CDI). Clin Microbiol Infect. 15. 1053–1066..



PL.3102

Warning
Hazardous ingredients: reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2Hisothiazol-3-one [EC no. 220-239-6] (3:1)
May cause an allergic skin reaction

Wear protective gloves. Avoid breathing vapour. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical attention. Dispose of contents and container in accordance with all local, regional, national and international regulations.

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