CoproStrip™ *C. difficile*

GDH+ Tox A+ Tox B

A rapid, one step test for the simultaneous qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B antigens in human faeces.

Instruction Manual

Test kit for 20 determinations
(Catalog No.41220)

For professional in vitro diagnostic use only
Store at 2-30°C. **Do Not Freeze**

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Intended Use: The CoproStrip GDH + Tox A + Tox B is a rapid chromatographic immunoassay combo card for the simultaneous qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human faeces that aids in the diagnosis of *C.difficile* infection.

SUMMARY AND EXPLANATION:

The gram-positive anaerobic bacillus *Clostridium difficile* is the leading causative agent of antibiotic-associated diarrhea and pseudomembranous colitiss (1). This pathogen is capable of causing disease that could be severe or fatal if not diagnosed on time and treated. Exposure to antibiotics is the major risk factor for *C. difficile* infection. Infection can develop if the normal gastrointestinal flora is disrupted by antibiotic therapy and a person acquires toxin-producing *C. difficile*, typically via the fecal-oral route (2). *C. difficile*'s key virulence factors are toxin A and toxin B (3, 4). These toxins show high sequence and functional homology. Toxin A has been described as a tissue damaging enterotoxin which attracts neutrophils and monocytes and toxin B as a potent cytotoxin that degrades the colonic epithelial cells (5). Most virulent strains produce both toxins, however, toxin A negative/toxin B positive strains are also capable of causing disease (6, 7). *Clostridium difficile* Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism (8, 9).

PRINCIPLE OF THE PROCEDURE

The CoproStrip™ C.difficile GDH+ Tox A+ Tox B is a qualitative immunoassay for detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human fecal specimens.
The membrane of the Test A is pre-coated with monoclonal antibodies against of *Clostridium difficile* (GDH) antigen, the membrane of the Test B is pre-coated with monoclonal antibodies against Toxin A of *Clostridium difficile* antigens and the membrane of the Test C is pre-coated with monoclonal antibodies against Toxin B of *Clostridium difficile* antigens on the test lines region. During testing, the sample reacts with the red colored particles coated with anti-GDH antibodies in the Test A and/or with anti-Toxin A antibodies in the Test B and/ or with anti-Toxin B antibodies in the Test C, which were pre-dried on the test strips. The mixture moves upward on the membrane by capillary action. In the case of a positive result in the Test A the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. In the case of a positive result in the Test B the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. In the case of a positive result in the Test C the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. The mixture continues to move across the membrane to the immobilized antibody places in the control band region. A green colored band always appears in the control lines and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

MATERIALS PROVIDED

- CoproStrip™ C.difficile GDH+ ToxA+ Tox B devices
- Instructions for use
- Sample collection vial with buffer

MATERIALS NOT PROVIDED

- Specimen collection container
- Disposable gloves
- Timer

WARNING 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 use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.

- The test must be carried out within 2 hours of opening the sealed aluminium pouch.

STORAGE AND STABILITY

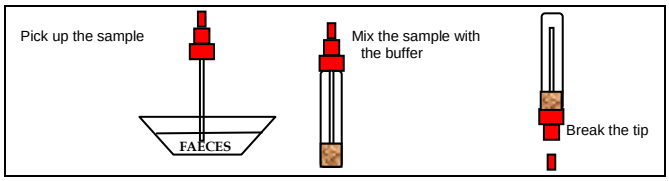
Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 24 hours prior to testing. For longer storage the specimen must be kept frozen at -20ºC/-4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

To process the collected stool samples (see illustration 1):
Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick into the faecal specimen to pick up the sample (approx. 125 mg). Close the vial with the buffer and stool sample. Vortex the vial for 15 seconds in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 125uL into the specimen collection vial with buffer.

Illustration 1



PROCEDURE

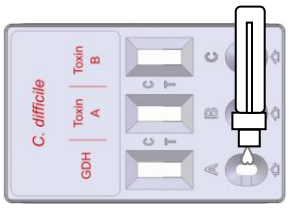
Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the Device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
3. Use a separate device for each sample. Dispense exactly 4 drops or 100 uL into each of the specimen wells (i.e. GDH well, Toxin A well, and Toxin B well, marked as S). Start the timer.
4. Read the results at **10 minutes** after dispensing the sample.

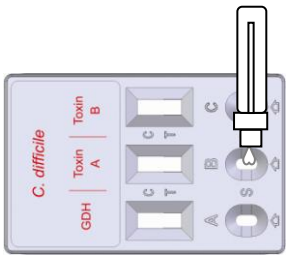
If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn't work, dispense a drop of diluents until seeing the liquid running through the reaction zone.

Illustration 2

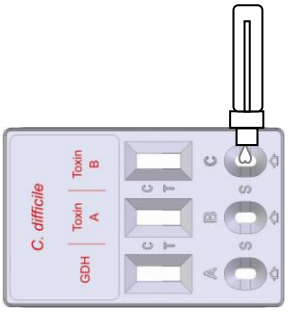
Add 4 drops of the diluted mixture in each circular window



GDH Test- well A



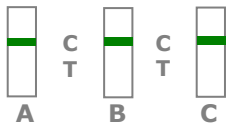
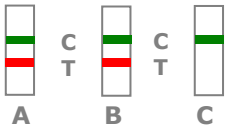
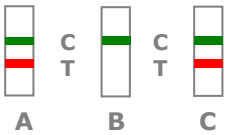
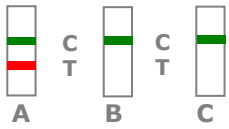
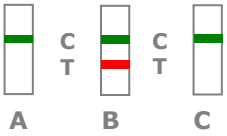
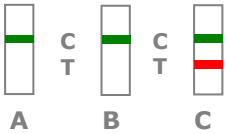
Toxin A Test- well B



Toxin B Test- well C

INTERPRETATION OF RESULTS

Illustration 3

1.	GDH, Toxin A and Toxin B of *Clostridium difficile* negative. 
2.	GDH and Toxin A positive. 
3.	GDH and Toxin B positive. 
4.	Toxin A and Toxin B positive. 
5.	GDH positive. Toxin A and Toxin B negative. 
6.	Toxin A positive. GDH and Toxin B negative. 
7.	Toxin B positive. GDH and Toxin A negative 
8.	Any other result Invalid result: either A, B or C, we recommend repeating the assay using the same sample with another combo test.

INVALID: Total absence of the green control band in one, two or the three Tests (A/B/C) regardless the appearance or not of the red test lines in one or both Tests (A/B/C). Notes: insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay using a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor. See illustration 3 above.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured band in the result line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

QUALITY CONTROL

Internal procedural controls are included in the test in the form of green lines appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS OF THE PROCEDURE

1. The CoproStrip™ C. difficile™ GDH+ Tox A+ Tox B test will only indicate the presence of GDH, Toxin A and/or B in the specimen (qualitative detection). Neither the quantitative value nor the rate of increase in antigens concentration can be determined by this test.
2. An excess of sample could lead to incorrect results (brown bands appear). In such a case, dilute the sample with the buffer and repeat the test.
3. Some stool samples can decrease the intensity of the control bands.
4. The test must be carried out within 2 hours of opening the sealed aluminum pouch.
5. 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 infection caused by *Clostridium difficile*.
6. This test provides a presumptive diagnosis of infection caused by *Clostridium difficile*. All results must be interpreted together with other clinical information and laboratory findings available to the physician and should be followed up with additional laboratory techniques.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

A study using stool samples from patients with diarrhea showed the following results: The sensitivity and specificity results using the CoproStrip™ C.difficile GDH+ Tox A+ Tox B test in comparison with other commercial immunoassays test (IC test: *C. DIFF QUIK CHEK Complete*® TechLab) were:
Sensitivity >99% and specificity >99%.

CROSS-REACTIVITY

An evaluation was performed to determine the cross reactivity of CoproStrip™ C. difficile GDH+Tox A+Tox B Device. There is no cross reactivity with common gastrointestinal microorganisms occasionally present in feces.

Campylobacter spp
E. coli O157:H7
Listeria monocytogenes
Helicobacter pylori
Shigella spp.
Staphilococcus aureus
Salmonella spp
Yersinia spp
Yersinia enterocolitica

BIBLIOGRAPHY

1. Cloud J, Kelly CP. Update on *Clostridium difficile* associated disease. Curr Opin Gastroenterol. 2007 Jan;23(1):4-9.
2. Owens RC Jr, Donskey CJ, Gaynes RP, Loo VG. Muto CA. Antimicrobial-associated risk factors for *Clostridium difficile* infection.Clin Infect Dis. 2008 Jan 15;46 Suppl 1:S19-31.
3. Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile colitis.* N Engl J Med. 1994 Jan 27;330(4):257-62
4. Voth DE, Ballard JD. *Clostridium difficile* toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005 Apr;18(2):247-63.
5. Savidge TC, Pan WH, Newman P, O'brien M, Anton PM, Pothoulakis C. *Clostridium difficile* toxin B is an inflammatory enterotoxin in human intestine. Gastroenterology. 2003 Aug;125(2):413-20.
6. Pituch H, van den Braak N, van Leeuwen W, van Belkum A, Martirosian G, Obuch-Woszczatyński P, Łuczak M, Meisel-Mikołajczyk F. Clonal dissemination of a toxin-A-negative/toxin-B-positive *Clostridium difficile* strain from patients with antibiotic-associated diarrhea in Poland. Clin Microbiol Infect. 2001 Aug;7(8):442-6.
7. Shin BM, Kuak EY, Yoo SJ, Shin WC, Yoo HM., Emerging toxin A-B+ variant strain of *Clostridium difficile* responsible for pseudomembranous colitis at a tertiary care hospital in Korea. Diagn Microbiol Infect Dis. 2008 Apr;60(4):333-7.
8. Lyerly DM, Barroso LA, Wilkins TD. Identification of the latex test-reactive protein of Clostridium difficile as glutamate dehydrogenase. J Clin Microbiol. 1991 Nov;29(11):2639-42.
9. Carman RJ, Wickham KN, Chen L, Lawrence AM, Boone JH, Wilkins TD, Kerkering TM, Lyerly DM. Glutamate dehydrogenase is highly conserved among Clostridium difficile ribotypes. J Clin Microbiol. 2012 Apr;50(4):1425-6.

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Symbols for IVD components and Reagents			
	Manufacturer	IVD	For *in vitro* diagnostic use only
EC REP	Authorized representative		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by
DIL	Sample diluent		