CoproStrip™ C. difficile GDH

A rapid one step test for the qualitative detection of *Clostridium difficile* glutamate dehydrogenase antigen in human feces..

INSTRUCTION MANUAL

Test kit for 20 determinations
(Catalog No.41222)

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

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Intended Use: The CoproStrip™ C. difficile GDH test is a one step rapid chromatographic immunoassay for the qualitative detection of *Clostridium difficile* glutamate dehydrogenase antigen in human feces specimens to aid in the diagnosis of *Clostridium difficile*.

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 through the fecal-oral route. This pathogen is the main cause related to antibiotic associated diarrhea and/or pseudomembranous colitis in hospitalized patients.
Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization. However, if the normal colonic flora is altered, resistance to colonization is lost. Thus, any factor associated with alteration of the normal enteric flora increases the risk of *C. difficile* colonization 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 the clinical manifestations, which range from mild, self-limited watery diarrhea to fulminant pseudomembranous colitis, toxic megacolon, and death. 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.. Most virulent strains produce both toxins, however, toxin A negative/toxin B positive strains are also capable of causing disease. All strains of *Clostridium difficile* produce high levels of Glutamate Dehydrogenase (GDH) enzyme by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

PRINCIPLE OF THE PROCEDURE

The CoproStrip™ C. difficile GDH test is a qualitative lateral flow immunoassay for the detection of GDH antigen in human feces samples. The membrane is pre-coated with monoclonal antibodies against GDH antigen on the test line region. During testing, the sample reacts with the red colored particles coated with anti-GDH antibodies, which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles conjugate migrate. In the case of a positive result, the specific antibodies present on the membrane will react with the conjugate and generate one red colored line. The mixture continues to move across the membrane to the immobilized antibody places in the control line region. A green colored line always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

MATERIALS PROVIDED

- 20 CoproStrip™ C. difficile GDH cassettes
- 20 Sample collection vials 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 after opening the sealed bag.

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) for 7 days prior to testing. For longer storage (maximum 1 year), the specimen must be kept frozen at -20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing.

M412222E 01-06/2021

PROCEDURE

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 four times into the fecal specimen to pick up the sample (approx. 125 mg).
Close the vial with the buffer and stool sample. vortex the vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 125 µL into the specimen collection vial with buffer.

Test Procedure (see illustration 2)
Allow the tests, stool samples and buffer to reach room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the CoproStrip™ C. difficile Toxin GDH cassette 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 cap of the vial.
3. Use a separate device for each sample. Dispense exactly 4 drops into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

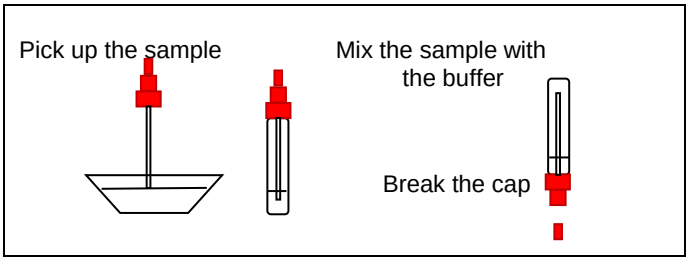
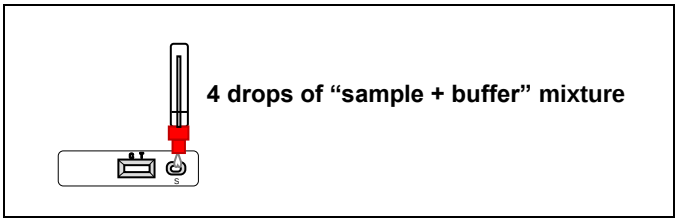
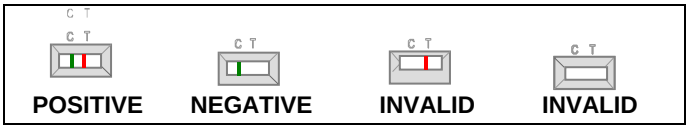


Illustration 2



INTERPRETATION OF RESULTS

Illustration 3



POSITIVE: Two lines appear across the central window, the **red** test line marked with the letter T and the **green** control line marked with the letter C.
NEGATIVE: Only one **green** line appears in the results window.
INVALID: Total absence of the green control line regardless the appearance or not of the red test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of reagents are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local distributor. See illustration 3.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red colored line (T) in the results window will vary depending on the concentration of GDH in the specimen. However, neither the quantitative value, nor the rate of increase in GDH can be determined by this qualitative test.

QUALITY CONTROL

Internal procedural controls are included in the test:
A green line (C) appearing in the results window. It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS OF THE PROCEDURE

1. *C. difficile* Ag *(*GDH) test will only indicate the presence of *Clostridium difficile* in the specimen (qualitative detection) and should be used for the detection of GDH antigen of *Clostridium difficile* in feces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause faulty results (brown-purple line appears). In this case, dilute the sample with the buffer and repeat the test.
3. Some stool samples can decrease the intensity of the control line.
4. This test provides a presumptive diagnosis of *Clostridium difficile* infection. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated must be based in the correlation of the results with further clinical observations.
5. Positive results confirm the presence of *Clostridium difficile*-GDH in fecal samples; nevertheless, it can be due to toxigenic or non-toxigenic strains of *Clostridium difficile*. A positive result should be followed up with additional laboratory techniques to determine the strain.
6. Bloody stool samples can contain components that may cause non-specific reactions in the test. Every bloody stool sample whose result is positive should be followed up with other techniques of diagnosis to confirm the result.
7. Mucous stool samples may cause non-specific reactions in the test- Every mucous stool sample whose result is positive should be followed up with other techniques of diagnosis to confirm the result.

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 diarrhea cases.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY
Two comparison studies performed in-house using the CoproStrip™ C. difficile GDH test and two other commercial immunoassays *C. DIFF QUIK CHEK Complete*® rapid test and the Wampole™ *C. Diff Chek*™-60, Techlab, ELISA test with 74 and 88 stool samples of symptomatic patients with

diarrhoea respectively, have obtained the following performance:

- *C. DIFF QUIK CHEK Complete*®,
  Sensitivity >99% and specificity >99%
- Wampole™ *C. Diff Chek*™-60
  Sensitivity >95% and specificity >99%

CROSS-REACTIVITY

In-house validation to determine the cross reactivity of the CoproStrip™ C. difficile GDH test with other gastroenteritis pathogens has shown that there is no cross reactivity with common gastrointestinal microorganisms present in feces as listed below:

- *Campylobacter* - *Listeria* - *Staphylococcus aureus*
- *E. coli* - *Salmonella* - *Yersinia*
- *H. pylori* - *Shigella*

BIBLIOGRAPHY

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2. VAISHNAVI, Ch., “Clinical spectrum & pathogenesis of *Clostridium difficile* associated diseases”. Indican J. Med. Res. 131, April 2010, pp 487-499
3. POUTANEN, S. M. et al. ”*Clostridium difficile*-associated diarrhoea in adults”, CMAJ, 171(1) July 2004, pp. 51-58.

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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		