CoproStrip™ C. difficile

Toxin A+B

A rapid one step test for the qualitative detection of Toxin A and B of *C. difficile* antigens in human feces.

INSTRUCTION MANUAL

Test kit for 20 determinations
(Catalog No.41223)

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 Toxin A+B test is a one step rapid chromatographic immunoassay for the simultaneous qualitative detection of *C. difficile* Toxin A and Toxin B antigens in human feces specimens to aid in the diagnosis of *C. difficile* infection.

SUMMARY AND EXPLANATION

Clostridium difficile is the principal pathogen related to antibiotic associated diarrhea and/or pseudomembranous colitis in hospitalized patients.
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. 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.

PRINCIPLE OF THE PROCEDURE

The CoproStrip™ C. difficile Toxin A+B test is a qualitative lateral flow immunoassay for the detection of Toxin A and Toxin B antigen in human feces samples. The membrane is pre-coated with monoclonal antibodies against Toxin A and/or Toxin B antigens on the test line region. During testing, the sample reacts with the red colored particles coated with anti-TOXIN A+B 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 Toxin A+B cassettes
- 20 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 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.

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. Shake 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 A+B 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 test 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

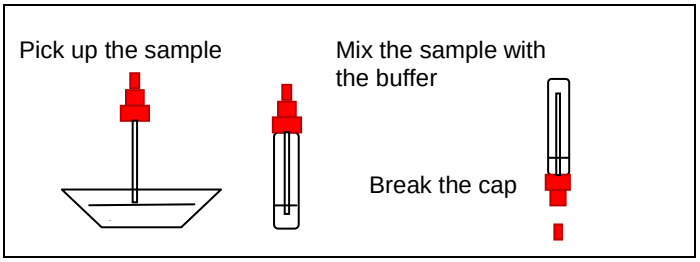
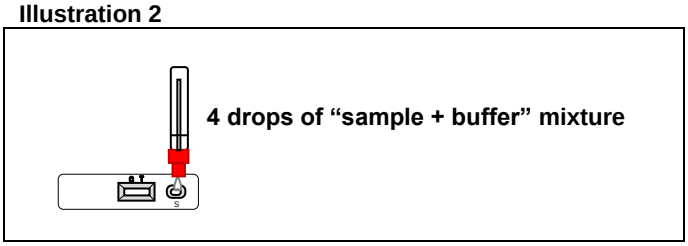


Illustration 2



INTERPRETATION OF RESULTS

Illustration 3

POSITIVE:

Toxin A positive: Two lines appear across the central window, a **red** test line marked with the letter T1 and a **green** control line marked with the letter C.

Toxin B positive: Two lines appear across the central window, a **red** test line marked with the letter T2 and a **green** control line marked with the letter C.

Toxin A+B positive: Three lines appear across the central window, the two **red** test lines (T1 and T2) and the **green** control line marked with the letter C.

NEGATIVE: Only one **green** line appears across the control line region marked with the letter C.

INVALID: Total absence of the green control coloured line regardless the appearance or not of the red test lines. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the 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 coloured test lines in the result line regions (T1 and T2) 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- A green line (C) appearing in the results window. It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS OF THE PROCEDURE

1. CoproStrip™ C. difficile Toxin A+B test will only indicate the presence of Toxin A and/or B ***(red and green lines)*** of *C. difficile* in the specimen (qualitative detection) and should be used for the detection of Toxins A and/or B antigens 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. 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 *Clostridium difficile* infection.
5. 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.

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

A comparison study performed in-house using the CoproStrip™ C. difficile Toxin A+B test in comparison with other commercial immunoassays test (*C. DIFF QUIK CHEK Complete*®, Techlab) with 50 stool samples of symptomatic patients with diarrhoea, has obtained the following performance:
Sensitivity >99% and specificity >99%.

CROSS-REACTIVITY

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

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

BIBLIOGRAPHY

1. 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
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		