C. difficile toxins A+B MonlabTest®

MO-10003 20 TESTS/MO-804017 20 TESTS IVD

One step test to detect C. difficile toxin A and toxin B

A rapid one step test for the qualitative detection of *Clostridium difficile* toxins A and B in human feces.
For professional in vitro diagnostic use only.

INTENDED USE

The C. difficile toxins A+B MonlabTest® is a rapid chromatographic immunoassay for the qualitative detection of toxins A and B in human feces specimens to aid in the diagnosis of *Clostridium difficile* infection.

SYNTHESIS

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.
Clostridium difficile is the principal pathogen 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.

PRINCIPLE

The C. difficile toxins A+B MonlabTest® is a qualitative lateral flow immunoassay for the detection of toxin A and toxin B in human feces samples. The membrane of the Test A is pre-coated with monoclonal antibodies against toxin A and the membrane of the Test B is pre-coated with monoclonal antibodies against toxin B on the test lines region. During testing, the sample reacts with the particle coated with anti-toxin A antibodies in the Test A and/or with anti-toxin B antibodies in the Test B which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugates and generate colored lines. A green colored band 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.

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 bag.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). 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.

MATERIALS PROVIDED	MATERIALS REQUIRED BUT NO PROVIDED
- 20 Tests - Instruction for use - 20 Specimen collection vial with buffer	- Specimen collection container - Disposable gloves - Timer

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 (2-4ºC) 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.

PROCEDURES

To process the collected stool samples (see illustration 1):
Use a separate specimen collection vial for each sample (with 1mL of the buffer). Introduce the swab or stick two or three times into the fecal specimen to pick up the sample (approx. 125 mg) and put into the testing tube or vial with buffer. Shake the testing tube or vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 125µL into the testing tube or vial with buffer.
Test Procedure (see illustration 2)
Allow the tests, stool samples and buffer to reach to room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until ready to perform the assay.
1. Remove the C. difficile toxins A+B MonlabTest® 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 top of the vial.
3. Use a separate device for each sample. Dispense 4 drops into the specimen well (S) of the Test A and dispense 4 drops into the specimen well (S) of the Test B using the same vial. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

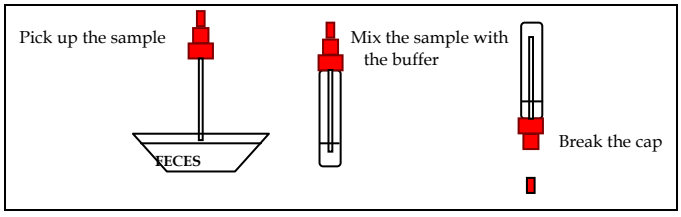
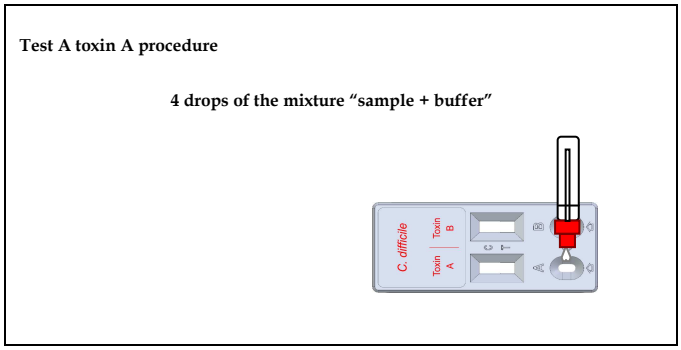


Illustration 2



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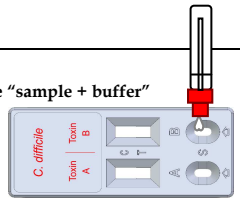
Test B toxin B procedure

4 drops of the mixture "sample + buffer"



INTERPRETATION OF RESULTS

Illustration 3

C T A B	Toxin A and toxin B negative	C T A B	Toxin A positive and toxin B negative
C T A B	Toxin A and toxin B positive	C T A B	Toxin A negative and toxin B positive

NEGATIVE:

Toxin A and toxin B negatives: two **green** lines appear across the windows, one in the Test A and one in the Test B marked with the letter C (control lines). See illustration 3.

POSITIVE:

Toxin A positive and toxin B negative: two lines appear across the window in the Test A (**red** test line marked with the letter T and a **green** control line marked with the letter C). Only one **green** control line appears across the window in the Test B, marked with the letter C. See illustration 3.

Toxin A negative and toxin B positive: Only one **green** control line appears across the window in the Test A. Two lines appear across the window in the Test B (**red** test line marked with the letter T and a **green** control line marked with the letter C). See illustration 3.

Toxin A and toxin B positives: two **green** lines and two **red** lines appear across the windows, one **green** and one **red** in Test A and one **green** and one **red** in Test B.

INVALID: A total absence of the **green** control colored band in one or both Tests (A/B) regardless the appearance or not of the **red** test line in one or both Tests (A/B). 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** colored lines in the result line regions (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:

- **Green** lines appearing in the control line regions (C). It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

1. The C. difficile toxins A+B MonlabTest® will only indicate the presence of toxins A and/or B in the specimen (qualitative detection) and should be used for the detection of toxins A and B in feces specimens only. Neither the quantitative value nor the rate of increase in antigens concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. 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*.
4. 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 associated diarrhea has been increased in frequency in recent years as a cause of nosocomial disease. The frequency and incidence of *Clostridium difficile* varies widely and is influenced by multiple factors including nosocomial outbreaks, patterns of antibiotic treatment and individual patient susceptibility.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

An evaluation was conducted comparing the results obtained using C. difficile toxins A+B MonlabTest® and C. DIFF QUIK CHEK Complete®, Techlab.

The C. difficile toxins A+B MonlabTest® was highly specific and sensitive (>99%) to detect toxins A and B compared with the results of that assay.

Cross-Reactivity

It was performed an evaluation to determine the cross reactivity of C. difficile toxins A+B MonlabTest®. There is not cross reactivity with common gastrointestinal pathogens, other organisms and substances occasionally present in feces.

- *Campylobacter spp.*
- *E.Coli O157:H7*
- *Helicobacter pylori*
- *Listeria monocytogenes*
- *Salmonella spp.*
- *Shigella spp.*
- *Staphilococcus aureus*
- *Yersinia spp.*

REFERENCES

- 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.
- Vaishnavi, Ch., "Clinical spectrum & pathogenesis of *Clostridium difficile* associated diseases". Indican J. Med. Res. 131, April 2010, pp 487-499.
- Clostridium difficile-associated diarrhea in adults, Susan M Poutanen, Andrew E. Simor.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For *in vitro* diagnostic use only
	Don't re-use		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by

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