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Rapid-VIDITEST

IVD

C. difficile Ag (GDH) Card/Blister

One step Clostridium difficile glutamate dehydrogenase antigen in human feces.

Instruction manual

INTENDED USE:

Rapid-VIDITEST C. difficile Ag (GDH) is a 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*.

INTRODUCTION:

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.
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.

PRINCIPLE:

Rapid-VIDITEST C. difficile Ag (GDH) is a qualitative immunoassay for the detection of GDH antigen in human feces samples. The membrane is pre-coated with 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 band region. A green colored band always appears in the control line (second line) and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

MATERALS PROVIDED:

- Rapid-VIDITEST C.difficile Ag (GDH) Card/Blister tests
- Instructions for use
- Specimen collection vial with buffer

MATERIALS REQUIRED BUT NO PROVIDED:

- Specimen collection container
- Disposable gloves
- Timer

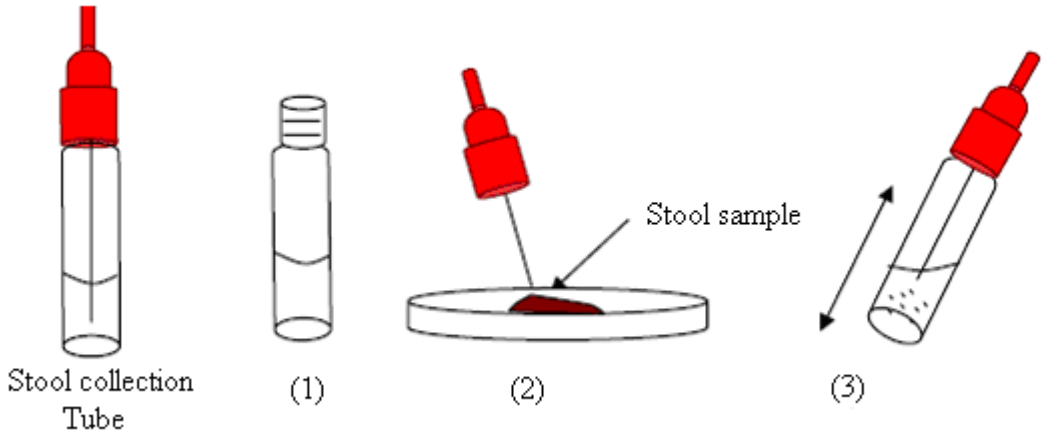
SPECIMEN COLLECTION AND PREPARATION:

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 (2-8ºC/36-46.4ºF) for 7 days 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:

Use a separate specimen collection vial for each sample.
Unscrew the cap of the vial and introduce the stick into the fecal specimen to pick up some sample (approx. 250 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 approx. 250 µL into the specimen collection vial with buffer.

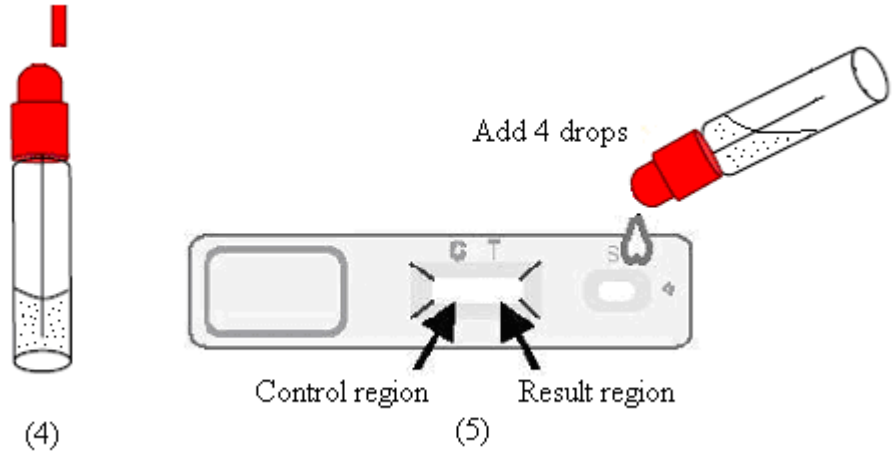


Test Procedure:

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

Test Procedure for Card test

1. Remove the Rapid-VIDITEST C.difficile Ag (GDH) Card from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure a good sample dispersion. Break off the cap of the vial (4).
3. Use a separate device for each sample. Dispense exactly 4 drops into the specimen well (S) (5). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.



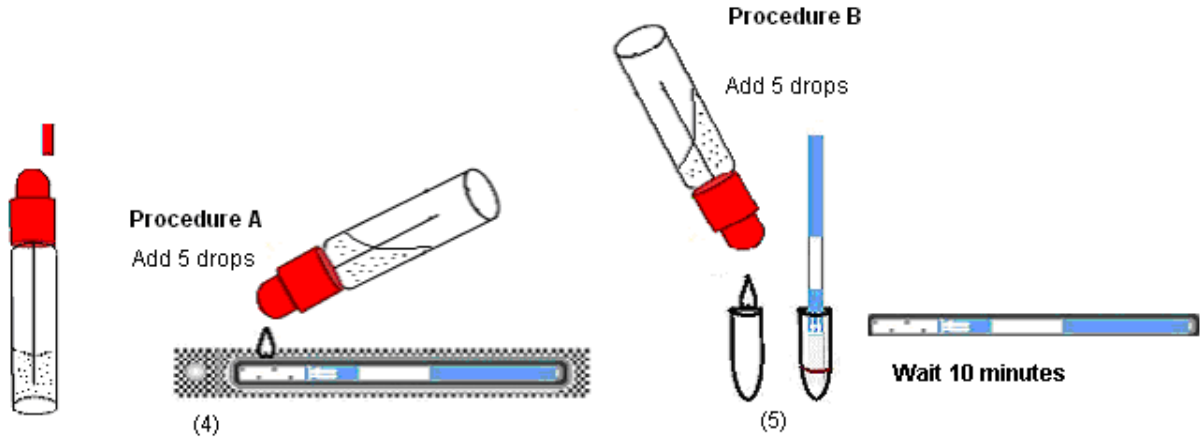
Test Procedure for Blister test

Procedure A: Using the blister test single pack as a card test:

1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil. Don't remove the test from the blister cavity and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Place the blister test single pack horizontally and identify it.
3. Dispense 5 drops of sample+buffer on the white end of the test (4). Start the timer. Read the result at 10 minutes after dispensing the sample.

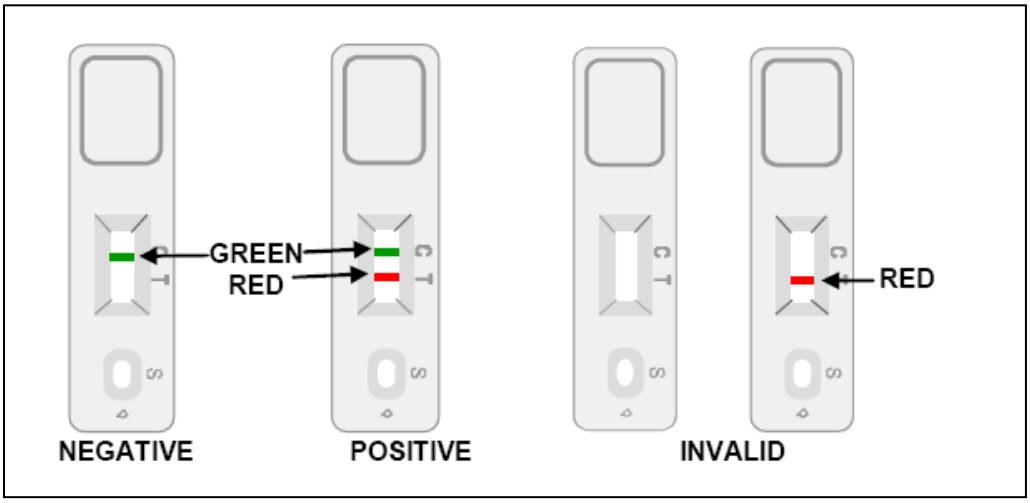
Procedure B: By immersion:

1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil.
2. Shake the specimen collection vial to assure good sample dispersion. Dispense 5 drops of sample+buffer in an identified vial and leave the test strip to stand vertically in the vial, taking care of not surpassing the limit of immersion indicated with the arrows (5). Start the timer. Read the result at 10 minutes.

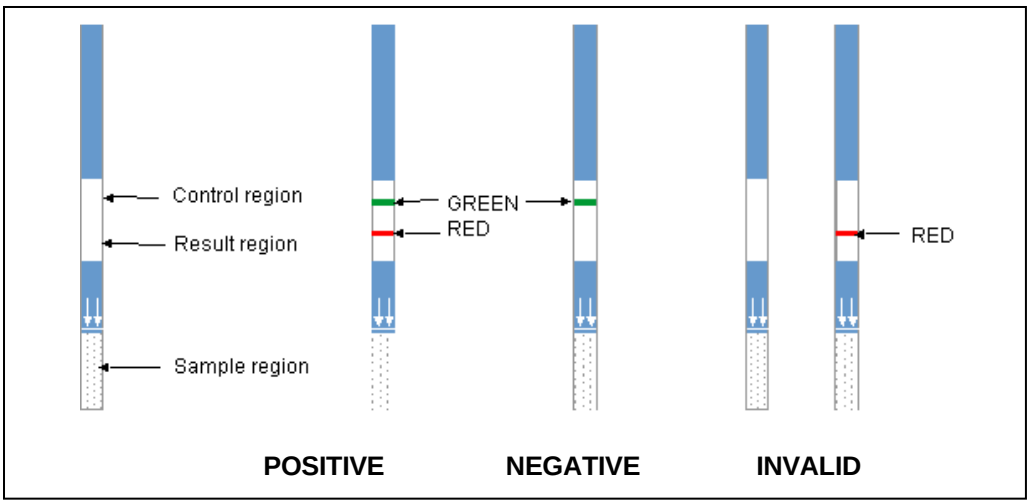


INTERPRETATION OF RESULTS:

CARD



BLISTER



POSITIVE: Two lines appears across the central window in the result line region, a **red** test line marked with the letter T and in the control line region, a **green** control line marked with the letter C.

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

INVALID: A total absence of the green control coloured band regardless the appearance or not of the red test line. 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 you local distributor.

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:
A green line appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS:

1. Rapid-VIDITEST C. difficile Ag (GDH) will only indicate the presence of *Clostridium difficile* in the specimen (qualitative detection) and should be used for the detection 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 wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. Some stool samples can decrease the intensity of the control line.
4. The test must be carried out within 2 hours of opening the sealed bag.
5. 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.
6. 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.

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

It was studied some stool samples from patients with diarrhea. The results showed using Rapid-VIDITEST C. difficile Ag (GDH) in comparison with other commercial immunoassays test (IC test: C. DIFF QUIK CHEK Complete® TechLab) were:
Sensitivity >99% and specificity >99%

Cross-reactivity

It was performed an evaluation to determine the cross reactivity of Rapid-VIDITEST C. difficile Ag (GDH). There is not cross reactivity with common gastrointestinal microorganisms occasionally present in feces: *Campylobacter spp., E.coli spp., H.pylori, Shigella spp., Salmonella spp., Yersinia spp.*

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.

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.

REFERENCES:

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.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

IVD	*In vitro* diagnostic device	LOT	Batch code
	Use by		Manufacturer

Last Revision: June 2012