I. Intended Use

The Biomerica *C. difficile Ag (GDH)* species test is a lateral flow immunoassay for the rapid determination of the presence of *Clostridium difficile* glutamate dehydrogenase antigen in human stool samples. The test is intended for professional *in vitro* use only.

II. 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.

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.

III. Principle of the Procedure

The *C. difficile* Ag (GDH) test utilizes the principle of non-competitive immunoassay. If GDH antigen is present in the sample at concentrations above the detection level, a GDH antigen specific antibody conjugated to colloidal gold binds to the antigen forming an antigen-antibody conjugate complex. An antibody which is immobilized in the Test region ("T") of the membrane is reactive to another epitope of the *C. difficile* antigen (GDH). The capture antibody will then react with the antigen-antibody conjugate complex. The captured complex will appear as a pink color band on the membrane. A distinct green color band will always appear at the Control region ("C") indicating that the test is functioning properly.

IV. Materials Provided

1. Test device in foil pouch
2. Stool collection vial with buffer
3. Instruction manual

V. Materials Required but Not Provided

Timer and Gloves

VI. Warnings and Precautions

Safety Precautions: Human stool should be handled as if capable of transmitting infectious agents. It is recommended that these specimens be handled using established good laboratory working practices. The test should be discarded in a proper biohazard container after testing.

For *in vitro* diagnostic use: Do not use the kit beyond the expiration date printed on the outside of the foil pouch. Discard all used test devices into a proper biohazard container. The test should remain in the sealed pouch until use. Do not use the test if pouch is damaged. The test must be carried out within 2 hours of opening the sealed pouch.

VII. Kit Storage

The *C. difficile* Ag (GDH) test can be stored refrigerated or at room temperature (2-30°C). The test is usable until the expiration date stamped on the foil pouch.

VIII. Quality Control

Although the *C. difficile* Ag (GDH) test contains an internal quality control (green color band in the Control region) good laboratory practice recommends the use of an outside control to ensure proper kit performance. Quality control samples should be tested according to the quality control requirements established by your laboratory.

IX. Test Procedure

Pre-treatment of the collected stool samples (see illustration 1):

Unscrew the cap of the provided stool collection vial filled with buffer. Using the stick on the cap collect about a pea size (150 mg) of solid sample. Place the stick back into the vial and close it. For liquid sample, aspirate about 150 µL into the vial and close it. Shake the vial thoroughly until the sample is dispersed well in buffer. Use a separate specimen collection vial for each sample being tested.

Test Procedure (see illustration 2):

Always allow the test devices, stool samples, and stool collection vials to reach room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the *C. difficile* Ag (GDH) test from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to ensure good sample dispersion. Break off the tip of the vial.
3. Dispense exactly 4 drops or 100 µL into the specimen well (S). Start the timer. Use a separate device for each sample
4. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

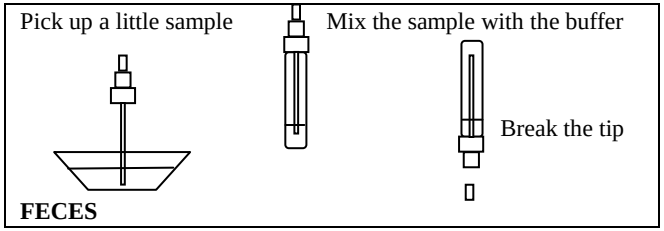
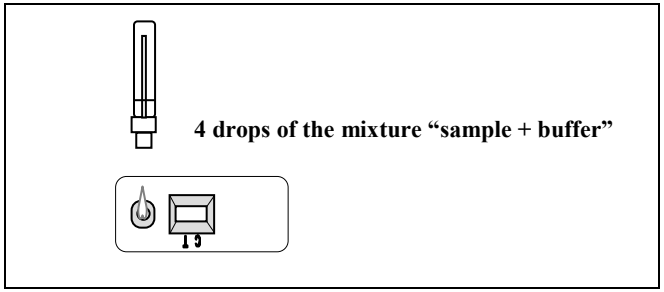
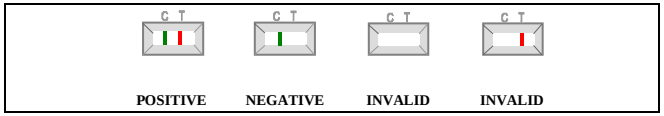


Illustration 2



X. Interpretation of Results

Illustration 3



POSITIVE: Two lines appear across the central window, in the Test region (a pink test line in the region marked with the letter T) and in the Control region (a green control line in the region marked with the letter C).

NEGATIVE: Only one band appears in the Control region (a green control line in the region marked with the letter C).

INVALID: A total absence of the control line regardless of the appearance of the test line. Note: Insufficient specimen volume, incorrect procedural techniques, or deterioration of the test materials 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 supplier.

The intensity of the pink colored band in the test 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.

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

XI. Performance Characteristics

Sensitivity and specificity

Stool samples were studied using the Biomerica *C. difficile* Ag (GDH) test yielding 99% sensitivity and >99% specificity.

Cross-reactivity

A study was performed to determine the cross reactivity of the Biomerica *C. difficile* Ag (GDH) test. No cross reactivity was found to common intestinal pathogens, other organisms and substances present in feces *(H. pylori, Yersinia spp, E. coli spp, Salmonella spp, Shigella spp, Campylobacter spp).*

XII. Limitations of the Test

1. The test is a qualitative method for stool samples only. It is not meant for quantitative analysis.
2. Excessive amount of the sample applied to the specimen well can make the background appear dark which may lead to a difficult interpretation of the result. In such a case, the sample should be diluted and the test repeated.
3. If a negative result is obtained and clinical symptoms persist, additional testing using other clinical methods and/or seeing a physician is recommended. A negative result does not preclude the possibility of *C. difficile* infection.
4. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

XIII. 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.

XIV. Ordering information

Ordering Information:

Telephone: 949-645-2111
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IVD

2°C – 30°C

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according to IVDD 98/79/ EC
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EZ-C. difficile

One-Step *C. difficile* Ag (GDH) Rapid Test

June 2012

REF **1010**

Immunoassay for
the qualitative determination of
Clostridium difficile
Glutamate Dehydrogenase (GDH)
antigen in human stool specimen

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