CLOSTRIDIUM DIFFICILE GDH

For *in Vitro* **diagnostic use**

Rapid immunochromatographic test for the qualitative detection of *Clostridium difficile* GDH common antigen in human feces.

I. INTRODUCTION AND INTENDED USE

CLOSTRIDIUM DIFFICILE GDH is a rapid immunochromatographic test for the qualitative detection of *Clostridium difficile glutamate dehydrogenase* antigen in human feces specimens to aid in the diagnosis of *Clostridium difficile* infection.
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.

II. PRINCIPLE OF THE TEST

CLOSTRIDIUM DIFFICILE GDH is a qualitative immunoassay for the detection of GDH antigen in human feces samples. The membrane is pre-coated with antibodies against GDH (red line) antigens on the test line region. During testing, the sample reacts with the 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 migrates. In the case of a positive result the specific antibodies present on the membrane will react with the conjugate and generate one colored line. The mixture continues to move across the membrane to the immobilized antibody placed 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.

III. REAGENTS AND MATERIALS

Each kit contains material for 25 determinations:
C. difficile GDH card (N.25) cassettes packaged with a desiccant in individual aluminum pouches
Diluent –Extraction buffer (25x1.0 mL tubes LIQ.EXTR) Sample Diluent of buffer containing proteins and preservative.
Instruction for use
Required materials (not supplied)
Specimen collection container, Disposable gloves and container, Plastic pipettes and Timer

IV. PRECAUTIONS

- For professional and *in Vitro* diagnostic use only.
- Do not use any of the kit contents after the expiration date and if not properly stored Do not mix kit components from different lots.
- Follow the instructions carefully
- The test is intended for single use only. Do not reuse the card for other tests
- The test must remain in the sealed pouch until use
- Follow the Good Laboratory Practices, wear protective clothing and disposable gloves, do not eat drink or smoke in the work area
- All specimens should be considered potentially hazardous and handled in the same way as infectious agents.
- The test should be eliminated in a special container in accordance with regulations in force

V. 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.

VI. 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.

VII. ASSAY PROCEDURE

Review “specimen collection” instructions. Do not open pouches until ready to perform the assay. Make sure that all reagents are at room temperature before beginning the assay.

Process the collected stool samples

Use a separate swab or stick, dropper and testing tube or vial for each sample. Unscrew the top of the extraction buffer tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen. Verify to transfer a small portion (150-250 mg) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 250 µl of each stool into an extraction tube. Mix carefully, then vortex 15 seconds.

Test Procedure

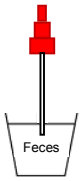
Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the C.DIFFICILE GDH from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample.
3. Break the cap of the tube

4. Dispense 4 drops or 100uL into the specimen well. Start the timer.
5. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

Pick up the sample



Mix the sample with the buffer and break the cap

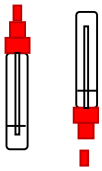
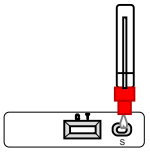


Illustration 2

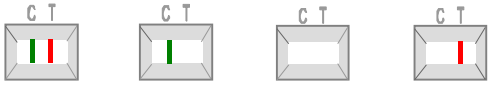


4 drops of the mixture “sample + buffer”

VIII. INTERPRETING THE RESULTS

POSITIVE: Two lines appear across the central window. A **red** line, (marked with the letter T, test line) and a **green** line (marked with the letter C, control line). See illustration 3.
NEGATIVE: Only one **green** line (control line) appears in the results window. See illustration 3.
INVALID: A 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, stop using the test kit and contact your local distributor. See illustration 3
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.

Illustration 3



Positive Negative Invalid

IX. CHARACTERISTICS

A) 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.
B) Sensitivity and Specificity
Some stool samples from patients with diarrhea were evaluated. The results using CLOSTRIDIUM DIFFICILE GDH in comparison with other commercial immunoassays test (IC test: C. DIFF QUICK CHEK Complete TechLab***)*** were:
Clinical Sensitivity >99% and Clinical specificity >99%
Analytical Sensitivity: 0.39 ng/mL
C) Cross reactivity
An evaluation to determine the cross reactivity of CLOSTRIDIUM DIFFICILE GDH test was performed. There was no cross reactivity with common gastrointestinal microorganisms occasionally present in feces: *Campylobacter, H. pylori, Salmonella spp, E. coli spp, Shigella spp and Yersinia spp.*

X. QUALITY CONTROL

Internal procedural controls are included in the test:
A green line (C) appearing in the results window confirms sufficient specimen volume and correct procedural technique.
Good laboratory practice includes the use of external control to ensure proper kit performance. Each day of testing (or according to the laboratory quality control procedures), a negative and a positive control containing a low level of CLOSTRIDIUM DIFFICILE GDH should be tested.

XI. LIMITS OF THE TEST

- C. difficile GDH test 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.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control line.
- The test must be carried out as soon as possible after opening the sealed bag.
- 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 and must be based in correlation with further clinical observations.
- Positive results confirm the presence of *Clostridium difficile* GDH in fecal samples. This can be due to toxigenic or non-toxigenic strains of *Clostridium difficile*. A positive result should be followed up by additional tests to determine the toxin production.

XII. BIBLIOGRAPHY : see the Italian version

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENTS **Ref. VC194065 (25 test)**
C. difficile GDH Card 25 items
Liq.Extr tubes 25 x 1.0 mL
Instruction for use 1 item

EDMA CODE 1501900200 CE IVD

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