



INSTRUCTIONS FOR USE

CLOSTRIDIUM DIFFICILE GDH PLUSRAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF *CLOSTRIDIUM DIFFICILE* GDH COMMON ANTIGEN IN HUMAN FECES.**1 – INTRODUCTION AND INTENDED USE**

Clostridium difficile GDH PLUS is a manual, rapid immunochromatographic test for the qualitative detection of *Clostridium difficile* glutamate dehydrogenase (GDH) in human feces specimens. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of Clostridium difficile infection. The kit contain a positive control.

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.

The use of a direct fecal GDH screen, together with a fecal Toxin AB test, could improve the diagnosis of Clostridium difficile infection.

2 - PRINCIPLE OF THE METHOD

Clostridium difficile GDH PLUS is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of Clostridium difficile Glutamate Dehydrogenase GDH in stool samples.

The strip consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against GDH and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-GDH) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming coloured conjugate complexes.

If the sample is positive, the antigen of the diluted sample reacts with the red-coloured conjugate complex (anti-GDH monoclonal antibodies-red polystyrene microspheres) which was previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-GDH antibodies present on the membrane (test line) capture the coloured conjugate and the red line will be visible. This band is used to interpret the result.

If the sample is negative, there is no GDH antigen presence and yet, the antigen may be present in a concentration lower than the detection limit value, for which the reaction will not take place with the red-coloured conjugate complex. The anti-GDH antibodies present on the membrane (test line) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red line will not appear. Whether the sample is positive or not, the mixture continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-specific protein antibodies present on the membrane will capture control green-conjugate complex and the control line will always appears. The presence of this green line serves as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Clostridium difficile GDH PLUS	Immunochromatographic test	VC194065P (25 tests)	25 sealed in foil pouch containing the device, with dessicant. 25 plastic tubes with dropper tip containing the extraction liquid, to use also like negative control (25 x 1 mL) 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN ₃ as preservative (0.5 mL) Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Plastic droppers, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- Clostridium difficile GDH PLUS is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- Read the instructions for use carefully before use the kit.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions. Do not use after expiration date.
- Do not use the test if the protective external box or the protective aluminium pouches are opened or damaged upon arrival.
- Do not use the test if desiccant material is not present or broken inside the aluminium pouch.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices, wear protective clothing, use disposal gloves or other personal protective equipment such as goggles and mask that will be considered necessary. Do not eat, drink or smoke in the working area.
- All the specimens should be considered potentially hazardous and handled according to the local or national safety regulations. Must be handled in the same manner as an infectious agent. Use proper infection control practices. These practices should include, but are not limited to, personal protective equipment (PPE), such as laboratory coat, surgical or appropriate mask, or face shield, disposable gloves and eye protection. Take necessary precautions during the collection, transport, storage, handling and disposal of the samples. Each sample must be correctly and unequivocally identified, in order to guarantee the correct traceability of the samples.
- Disposable gloves, extraction buffer, test tubes, and used devices in a propre biohazard container. These containers should be discarded in accordance with local or national laws and regulations.





- Each test device and each extraction buffer vial are for single use only, to avoid contamination errors.
- Clean up spills thoroughly using an appropriate disinfectant.
- Reagents contain preservatives (<0.1% sodium azide). Avoid any contact with skin or mucous membrane. In accordance with Regulation (EC) N° 1907/2006 (REACH), Clostridium difficile GDH PLUS do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) N° 1272/2008 (CLP) or which are in concentrations higher than the value established in the mentioned regulation for their declaration. Material Safety Data Sheet is not included with this device.
- The presence of yellow lines in the results window (control and test line zone) that are visible before using the test are completely normal. That not means failure on test functionality.
- Visual interpretation of the results must be done by professional user without problems in colour interpretation.
- The Certificates of Analysis of the product are available on the website www.masciabrunelli.it.
- If the device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case it is recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Do not freeze.

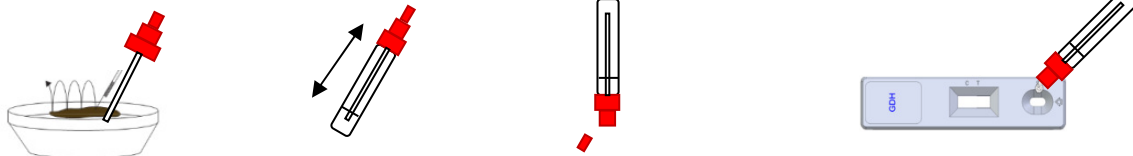
7 – SPECIMENS AND PREPARATION

Stool specimen should be collected as soon as possible after onset of symptoms. Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8°C) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C. Freezing and thawing cycles are not recommended. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize stool sample as thoroughly as possible prior to preparation. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended.

Samples preparation

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in *four* different places of the same stool specimen and cover the stick's screw to pick up the sample. Verify to transfer a small portion of stool (approx. 50 mg). Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Wait at least 3 minutes. Repeat the operations just to obtain a dark yellow-brown solution, if necessary.

The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow. For **liquid samples**, add approx. 125µL in the stool collection tube using a micropipette. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion.



8 - TEST PROCEDURE

Allow the tests, stool samples, buffer and control 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 test card from the protective pouch. Identify the plastic cassette with the patients data.
2. Gently shake the test tube containing the sample under investigation. Brake the tip of the test tube.
3. Squeeze 3 drops of the extracted mixture into the sample well "S" of the card. Avoid adding solid particles with the liquid.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded 10 minutes.

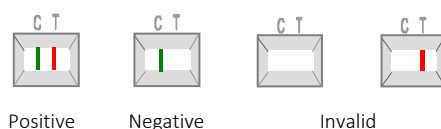
If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn't work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

Controls Procedure

Add the requested volume 3 drops (about 100 µL) of Positive/Negative Controls into the sample well of the cassette and Read the test results 10 minutes.

9 - READING AND INTERPRETATION

Interpret the results as follow:



Positive

Negative

Invalid





	GDH	Interpretation of results
1.	- GREEN	There is no <i>Clostridium difficile</i> presence. No infection caused by <i>Clostridium difficile</i> . Negative result.
	+ RED-GREEN	There is <i>Clostridium difficile</i> presence. <i>Clostridium difficile</i> infection, which might mean milder or severe (colitis) diarrhoea caused by <i>C. difficile</i> or asymptomatic patient, carrier of disease.
3.	OTHER RESULTS	Invalid result, we recommend repeating the assay using the same sample with another test.

INVALID: Total absence of any control coloured line (GREEN) regardless the appearance or not of the test line (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured band in the test line (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

10 - INTERNAL QUALITY CONTROL

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

11 – 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. In addition, nearly 223.900 people in the United States required hospital care for *C. difficile* and at least 12.800 people died in 2017. Although U.S. outpatient antibiotic prescription rate decreased from 2011 to 2016, at least 30% of outpatient antibiotic prescription are estimated to be unnecessary, which highlights the need to improve outpatient prescribing.

12 - PERFORMANCES CHARACTERISTICS

A. Analytical sensitivity (detection limit)

Detection limit for *C. D. GDH* is: 0,39 ng/mL. of *Clostridium difficile* antigen GDH.

B. Clinical sensitivity and specificity

A study was performed on faeces samples, using the *Clostridium Difficile GDH PLUS* versus Evaluation Criteria. Evaluation Criteria consisted on the evaluation in parallel two rapid vs other rapid test from the competitor; the discrepant results were confirmed by qPCR technique. The results were with a confidence interval of 95%:

Sensitivity 96,6% (90,5-99,3%) Specificity 99,4% (96,6-100,0%) PPV 98,9% (93,8-100,0%) NPV 98,2% (94,7-99,6%)

C. Cross reaction

An evaluation was done to determine the cross reactivity of *Clostridium difficile GDH PLUS*. There is not cross reactivity against gastrointestinal pathogens, other organism, substances and/or faecal markers occasionally present in faeces: *Adenovirus, Astrovirus, human and pig haemoglobin; bovine lactoferrin; bovine transferrin, Clostridium difficile Toxin A, Clostridium difficile Toxin B, Clostridium perfringens, Clostridium bifermentans, Clostridium Butyricum, Clostridium Haemolyticum, Clostridium Novyi, Clostridium Tetani, Clostridium Septicum, Coronavirus, Cryptosporidium parvum, Entamoeba histolytica, Helicobacter pylori, Campylobacter spp, E.ColiO157:H7, E.ColiO111; E.ColiO26; Giardia lamblia; Influenza A and B, Legionella pneumophila; Listeria monocytogenes, Norovirus GI and GII; Peptostreptococcus anaerobius, Respiratory Syncytial Virus, Rotavirus, Salmonella enteritidis/paratyphi A/typhi/typhimurium, Shigella boydii/dysenteriae/flexneri/somei, Staphylococcus aureus, Streptococcus pneumococcal, Streptococcus pyogenes; Yersinia enterocolitica O:3/O:9.*

A specificity assay was performed for CLOSTRIDIUM DIFFICILE GDH PLUS. This test could detect the following antigens: *C. sporogenes* (CECT 485) and *C. botulinum* (CECT 551).

In this specificity assay, *Clostridium sordelli* (ATCC 9714) was evaluated and no cross reactivity was found. In few intercomparison studies CLOSTRIDIUM DIFFICILE GDH PLUS have a positive signal with this pathogen, but the nature of this positive result could not be established.

D. Interferences

It was performed an evaluation to determine the possible interferences of CLOSTRIDIUM DIFFICILE GDH PLUS. There are no interferences against the substances and/or fecal markers occasionally present in feces:

Exogenous interferences

Metronidazole, Ibuprofen, Almagata, Amoxicillin, Ampicillin, Paracetamol, Fosfamyacin, Mercaptopurine, Oseltamivir, Metamizole, Acetylcysteine, Biotine, Amantadine, Prednisone, Dexametopfen trometamol, Sore Throat Phenol spray, Ribavirin, Omeprazole, Levofloxacin, Tobramycin, Codeine, Naso GEL, Ciprofloxacin, Mupirocin, Benzocaine, CVS Nasal Spray, Rifampicin, Fluticasone Propionate, Cloperastine, Oxymetazoline, Phenoxyethylpenicillin potassium, Carbocysteine, CVS Nasal Drops (Phenylephrine), Ambroxol hydrochloride, Mercaptopurine, Loratadine, Macrogol 3350, Dexchlorpheniramine, Phenylpropanolamine, Lysine Carbocysteinate, Homeopathic, Ebastine, Loperamide hydrochloride, Hydroxyzine dihydrochloride, Acetyl Salicylic, Heparin, Lorazepam.

Endogenous interferences

Human haemoglobin, Human transferrin, Human calprotectin, Mucine, Human blood, Human lactoferrin.

E. Repeatability and Reproducibility

It was performed a study of repeatability and reproducibility using different internal samples, negative and positive. There are no differences observed within the evaluations.

13 - LIMITATIONS OF THE METHOD

- *Clostridium difficile GDH PLUS* should only be used on human faecal samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained. This test cannot determine either the quantitative value or the rate of increase in the concentration of GDH antigen of *C. difficile*.
- The test should remain in the sealed pouch until use, and the test must be carried out within 2 hours of opening the sealed bag.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the extraction liquid and repeat the test.





- The intensity of the test line may vary from very strong at high antigens concentration to faint when the antigens concentrations is close to the detection limit value of the test.
- 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.
- 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 (toxigenic culture) to determine the strain. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigen concentration in the stool samples was too small (lower than the detection limit). If clinical symptoms persist, a Clostridium difficile determination should be carried out on a sample from an enrichment culture.
- Mucous and/or bloody stool samples could cause non-specific reactions in the test. Mucous and/or bloody stool samples whose result is positive should be followed up with other techniques to confirm the result.

14 - 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". Indian J. Med. Res. 131, April 2010, pp 487-499
3. Ofori E, Ramai D, Dhawan M, Mustafa F, Gasperino J, Reddy M. Community-acquired Clostridium difficile: epidemiology, ribotype, risk factors, hospital and intensive care unit outcomes, and current and emerging therapies. J Hosp Infect. 2018;99(4):436-42.
4. Guh AY, Mu Y, Winston LG, Johnston H, Olson D, Farley MM, et al. Trends in U.S. Burden of Clostridioides difficile Infection and Outcomes. N Engl J Med. 2021;382(14):1320-30.

TABLE OF APPLICABLE SYMBOLS

	In Vitro Diagnostic Medical Device		Temperature limitation		Batch code (DXXX)		Manufacturer		Keep dry		Unique device identifier
	Consult Instructions for use		Use by (year/month)		Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 5	Updated layout and content; alignment to the Italian version revision index	2022/11
Instructions for Use (IFU) - Revision 6	Removal of obsolete classifications	2023/03
Instructions for Use (IFU) - Revision 7	Update content	2023/11

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

