INSTRUCTIONS FOR USE

COMBI GDH-TOXIN A+B

IMMUNOCROMATOGRAPHIC RAPID TEST FOR DETECTION OF *CLOSTRIDIUM DIFFICILE* GDH COMMON ANTIGEN, TOXIN A AND TOXIN B IN HUMAN STOOL SPECIMEN

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

COMBI GDH-TOXIN A+B is a immunochromatographic assay for the simultaneous qualitative detection of *Clostridium difficile* GDH common antigen, toxin A and toxin B in human feces, including Positive Control. The test offers a simple and highly sensitive screening assay to make a presumptive 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.

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

COMBI GDH-TOXIN A+B is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the simultaneous qualitative detection of *Clostridium difficile* GDH common antigen, toxin A and toxin B in stool samples.

The device consists of 3 different strips: one for the determination of Toxin A, one for the determination if Toxin B of *C. difficile* and one for the determination of GDH common antigen. The strips consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against GDH (on A strip), against Toxin A (on B strip) and against Toxin B (on C strip) and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The sample absorbent pad is sprayed with test label solution containing mouse monoclonal antibodies anti-GDH (A strip), anti-Toxin A (B strip), anti-Toxin B (C strip) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming two coloured conjugate complexes.

If the sample is GDH positive, the antigens of the diluted sample react with the red-coloured conjugates complex (anti-GDH monoclonal antibodies-red polystyrene microspheres) in the strip A, if the sample is Toxin A positive, the antigens of the diluted sample react with the red-coloured conjugates complex (anti-Toxin A monoclonal antibodies-red polystyrene microspheres) in the strip B, and if the sample is Toxin B positive, the antigens of the diluted sample react with the red-coloured conjugated complex (anti-Toxin B monoclonal antibodies-red polystyrene microspheres) in strip C which were 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 of strip A (test line), the anti-Toxin A antibodies present on the membrane of strip B (test line) and the anti-Toxin B antibodies present on the membrane of strip C (test line) capture the coloured conjugate and the red line will be visible in the strips. These bands are used to interpret the result. If the sample is negative, there is no GDH, Toxin A and Toxin B presence and yet, the antigens may be present in a concentration lower than the detection limit value, for which the reaction will not take place with any red-coloured conjugate complex. The anti-GDH, the anti-Toxin A and the anti-Toxin B antibodies present on the membranes (test lines) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red lines will not appear. Whether the sample is positive or not, in the strips, the mixture continues to move across the membranes to the immobilized specific antibodies placed in the control lines. The anti-specific protein antibodies present on the membranes will capture control green-conjugate complex and all teh control lines will always appear. The presence of these green lines serve 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
COMBI GDH-TOXIN A+B	Immunochromatographic test	**VC194070P** (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) 10 plastic pipettes. Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- COMBI GDH-TOXIN A+B 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 googles and mask that will be considered necessary. Do not eat, drink or smoke in the working area.

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- 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) Nº 1907/2006 (REACH), COMBI GDH-TOXIN A+B do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) Nº 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 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

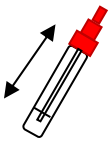
Stool specimen should be collected as soon as possible after onset of symptoms. Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC. Freezing 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.

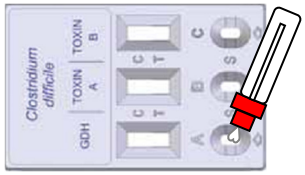
Liquid or Semi-Solid Stools: Using a separate pipette (included with the kit) for each stool, draw stool of the sample itself. Dispense 6-7 drops (about 125 µL) of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds.

Care should be taken when pipetting semi-solid stool. The addition of less than indicated of stool may cause a false-negative test. The addition of more than indicated of stool may cause invalid results due to restricted sample flow.

Formed / Solid Stools: 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.



8 - 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 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.
3. Brake the tip of the test tube and squeeze 3 drops of the extracted mixture into the sample well "A", 3 drops into the sample well "B" and 3 drops into the sample well "C" of the card.
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.

Procedure for the controls
Add the requested volume (3-4 drops (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:

	A (GDH)	B (Toxin A)	C (Toxin B)	Interpretation of results
1.	- green	- green	- green	GDH, Toxin A and Toxin B of *Clostridium difficile* negative.
2.	+ green/red	+ green/red	+ green/red	GDH, Toxin A and Toxin B of *Clostridium difficile* positive.
3.	+ green/red	+ green/red	- green	GDH and Toxin A of *Clostridium difficile* positive. Toxin B negative.
4.	+ green/red	- green	+ green/red	GDH and Toxin B of *Clostridium difficile* positive. Toxin A negative.
5.	+ green/red	- green	- green	GDH antigen positive. Toxin A and Toxin B negative.
6.	- green	+ green/red	+ green/red	If the result appears it must be repeat the test using a fresh sample. If results are again positive for Toxin A and B and negative for GDH, the sample should be considered positive for Toxin A and B.
7.	- green	+ green/red	- green	If the result appears it must be repeat the test using a fresh sample. If results are again positive for Toxin A and negative for GDH, the sample should be considered positive for Toxin A.
8.	- green	- green	+ green/red	If the result appears it must be repeat the test using a fresh sample. If results are again positive for Toxin B and negative for GDH, the sample should be considered positive for Toxin B.
9.	Any other results			Invalid result either A, B or C, 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 lines (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 bands in the test lines (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 GDH C.D. is 0,39 ng/mL
Detection limit for C. D. Toxin A is: 2 ng/mL.
Detection limit for C. D. Toxin B is: 3,12 ng/mL.

B. Clinical sensitivity and specificity (Toxin A+B)
A study was performed on faeces samples, using the COMBI GDH-TOXIN A+B versus Evaluation Criteria. For strip A: GDH, the Evaluation Criteria consisted on the evaluation in parallel of our rapid test, vs other rapid test from the competitor; the discrepant results were confirmed by qPCR technique.
For strip B and C: Toxin A and B, the Evaluation Criteria consisted on the evaluation in parallel of our rapid test vs other rapid test from the competitor: the discrepant results were confirmed by qPCR technique. The results were as follows:

GDH:	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%)
Toxin A:	Sensitivity 98,1% (89,7-100,0%)	Specificity 100,0% (98,2-100,0%)	PPV 100,0% (93,0-100,0%)	NPV 99,5% (97,2-100,0%)
Toxin B:	Sensitivity 90,9% (78,3-97,5%)	Specificity 100,0% (98,2-100,0%)	PPV 100,0% (91,2-100,0%)	NPV 98,1% (95,2-99,5%)

C. Cross reaction
It was performed an evaluation to determine the cross reactivity of COMBI GDH-TOXIN A+B. There is not cross reactivity with common intestinal pathogens, other organisms, substances and/or fecal markers occasionally present in feces.
For test A: GDH:
Adenovirus, Astrovirus, bovine and pig haemoglobin; bovine lactoferrin; bovine transferrin, Clostridium difficile Toxin A/Toxin B, Clostridium perfringens, Clostridium bifermentans, Clostridium Butyricum, Clostridium Haemolyticum, Clostridium Novyi, Clostridium Tetani, Clostridium Septicum, Coronavirus, Cryptosporidium parvum, Entamoeba histolytica, Helicobacter pylori, Campylobacter coli/jejuni, E.Coli O157:H7, E.Coli O111; E.Coli O26; 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/sonnei, Staphylococcus aureus, Streptococcus pneumococcal, Streptococcus pyogenes; Yersinia enterocolitica O:3/O:9.

A specificity assay was performed for COMBI GDH-TOXIN A+B (test A). This test could detect the following antigens: C. sporogenes (CECT 485) and C. botulinium (CECT 551).
In this specificity assay, Clostridium sordelli (ATCC 9714) was evaluated and no cross reactivity was found. In few intercomparison studies COMBI GDH-TOXIN A+B (test A) have a positive signal with this pathogen, but the nature of this positive result could not be established.

For test B: Toxin A

Adenovirus, Astrovirus, bovine and pig haemoglobin; bovine lactoferrin; Clostridium difficile GDH/Toxin B, Clostridium perfringens, Coronavirus, Cryptosporidium parvum, Entamoeba histolytica/dispar, Helicobacter pylori, Campylobacter coli/jejuni, E.Coli O157:H7, E.Coli O111; E.Coli O26; Giardia lamblia; Influenza A and B, Legionella pneumophila; Listeria monocytogenes, Norovirus GI and GII, Respiratory Syncytial Virus, Rotavirus, Salmonella enteritidis/paratyphi A/typhi/typhimurium, Shigella boydii/dysenteriae/flexneri/sonnei, Staphylococcus aureus, Streptococcus pneumococcal, Streptococcus pyogenes; Yersinia enterocolitica O:3/O:9.

For test C: Toxin B

Adenovirus, Astrovirus, bovine and pig haemoglobin; bovine lactoferrin; Clostridium difficile GDH/Toxin A, Clostridium perfringens, Coronavirus, Cryptosporidium parvum, Entamoeba histolytica/dispar, Helicobacter pylori, Campylobacter coli/jejuni, E.Coli O157:H7, E.Coli O111; E.Coli O26; Giardia lamblia; Influenza A and B, Legionella pneumophila; Listeria monocytogenes, Norovirus GI and GII, Respiratory Syncytial Virus, Rotavirus, Salmonella enteritidis/paratyphi A/typhi/typhimurium, Shigella boydii/dysenteriae/flexneri/sonnei, Staphylococcus aureus, Streptococcus pneumococcal, Streptococcus pyogenes; Yersinia enterocolitica O:3/O:9.

D. Interferences

It was performed an evaluation to determine the possible interferences of COMBI GDH-TOXIN A+B. There are no interferences against the substances and/or fecal markers occasionally present in feces:

Exogenous interferences

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

Endogenous interferences

Human haemoglbin, 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

- COMBI GDH-TOXIN A+B 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, Toxin A and/or B 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 determine the presence of GDH, Toxin A and Toxin B of *Clostridium difficile* in fecal samples. 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. Lierly, D,M, H.C. Krivan, and T.D. Wilkins. 1988. Clostridium difficile : its disease and toxins. Clin. Microbiol. Rev. 1:1-18.
2. 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
3. Vaishnmavi, Ch., "Clinical spectrum & pathogenesis of Clostridium difficile associated diseases". Indican J. Med. Res. 131, April 2010, pp 487-499
4. Poutanen, S. M. et al. "Clostridium difficile-associated diarrhoea in adults", CMAJ, 171(1) July 2004, pp. 51-58.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	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 4	Updated layout and content; alignment to the italian version revision index	2023/03
Instructions for Use (IFU) - Revision 5	Update content	2023/11

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

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