CLOSTRIDIUM DIFFICILE TOXIN A+B CARD

For *in Vitro* **diagnostic use**

Immunochromatographic rapid test for the detection of *Clostridium Difficile* Toxin A and Toxin B in faeces

I. INTENDED USE

Clostridium Difficile Toxin A+B Card is a lateral flow, immunochromatographic rapid test for the qualitative detection of *Clostridium difficile* Toxin A and Toxin B in human faeces.

II. PRINCIPLE

The test device consists of a plastic housing containing two different sticks for the detection of *C.difficile* Toxin A or *C.difficile* Toxin B. A coloured anti *C.difficile* Toxin A or *C.difficile* Toxin B monoclonal antibody latex conjugate is placed at the left end of the membrane.
After collection in a tube containing the extraction solution, the faeces sample is dissolved and few drops of this extract are added into each well of the reaction device.As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the Toxin A or Toxin B antigen (when present in the sample), forming an antibody antigen complex. This complex binds to the monoclonal anti-Toxin A or Toxin B antibody in the positive reaction zone, producing a red coloured band.In the absence of Toxin A or Toxin B, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device, past the positive reaction zone and control zone.
Unbound conjugate binds to the reagent in the control zone producing a green coloured band demonstrating that the reagents are functioning correctly.

III. REAGENTS AND MATERIALS

Each kit contains everything needed to perform 25 tests.
- 25 devices for immunochromatographic reaction
- 25 vial with 1 ml of Extraction Buffer
- 1 Instructions leaflet.

IV. STORAGE AND STABILITY

Storage the kit at room temperature (between 2°C and +30°C). The kit is stable until the expiry date stated on the package label. The test must remain in the sealed pouch until use. Do not freeze.

AUXILIARY REAGENTS (Not supplied with this kit) *Positive and Negative control (Mascia Brunelli Ref. UD80360*)

V. PRECAUTIONS

- This test is designed for *in vitro* diagnostic use and professional use only.
- Read carefully instructions leaflet before using this test.
- Do not use beyond the expiry date stated on the package label.
- All reagents and materials coming in contact with potential infectious specimens must be treated with appropriate disinfectants or autoclaved at 121°C for at least one hour.
- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples. Do not eat, drink or smoke in the area where specimens and kit reagents are handled. Avoid any contact between hands and eyes or nose during specimen collection and testing.
- Do not use a test from a damaged protective wrapper.
- The test must be carried out within 2 hours of opening the sealed bag.

VI. SPECIMEN COLLECTION AND PREPARATION

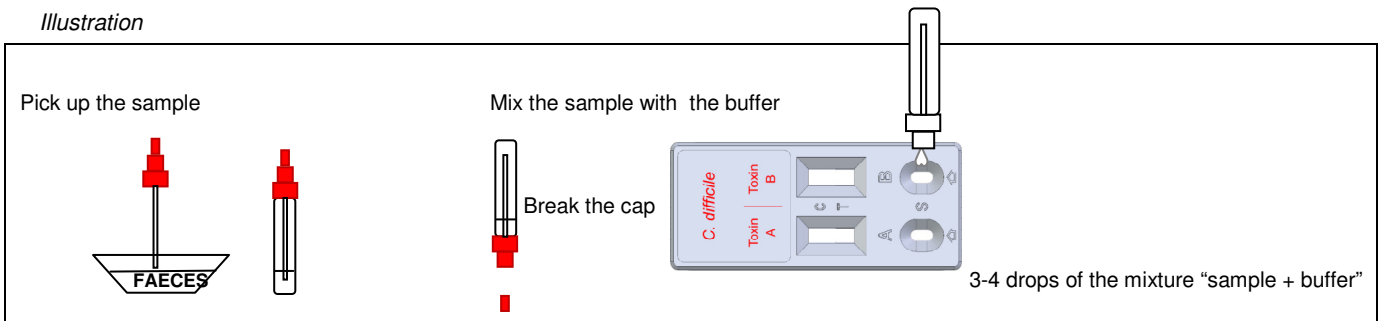
Stool specimen should be collected as soon as possible after onset of symptoms. Do not collect specimens in containers having media, preservatives, animal serum or detergents, as any of these may interfere with the test.
Diluted samples may be stored at +2°C to +8°C for 3 days without interference with assay performance. For long term storage of undiluted specimens, storage at –20°C or colder is recommended. Repeated freezing and thawing of samples is not recommended and may cause erroneous results.
Unscrew the top of the extraction 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 (approximately 125 mg-3 mm diameter) of stool. 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. 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.

Liquid or Semi-Solid Stools

Using a separate pipette (included with the kit) for each stool, draw stool of the sample itself. Dispense 5-6 drops (200 µL) of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds

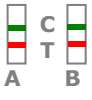
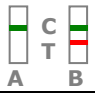
VI. ASSAY PROCEDURE

Illustration



1. Let all the reagents warm up to room temperature before proceeding with the test.
2. Stir thoroughly to homogenize the solution the vial with sample and extraction buffer.
3. Break the dropper of the vial and dispense 3-4 drops (100 µL) into the card’s well.
4. Read the test results 10 minutes after addition of the sample to the device

VII. READING TEST RESULTS

1.		Toxin A and Toxin B of *Clostridium difficile* negative.
2.		Toxin A and Toxin B of *Clostridium difficile* positive.
3.		Toxin A of *Clostridium difficile* positive. Toxin B of *Clostridium difficile* negative.
4.		Toxin A of *Clostridium difficile* negative. Toxin B of *Clostridium difficile* positive.
5.	Any other result	Invalid result: either A or B, we recommend repeating the assay using the same sample with another combo test.

VIII. QUALITY CONTROL
Internal procedural controls are included in the test:
Green lines appearing in the control lines regions (C). It confirms sufficient specimen volume and correct procedural technique.

IX. PERFORMANCES CHARACTERISTICS

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

C. difficile TOXIN A - Analytical sensitivity
The performance of the test has been assayed, using a range diluted solutions prepared from a commercially available purified toxin A antigen. The test is able to detect a concentration of 0.5-1 ng/mL.

C. difficile TOXIN B - Analytical sensitivity
The sensitivity of the test has been evaluated using a range of diluted solutions prepared from a commercially available purified C.D. Toxin B antigen. Under these conditions, the detection limit of the test has been found to be 0.39-1.56ng/mL.

C. difficile TOXIN A+B - Diagnostic sensitivity and specificity
A study was performed on feces samples, using the Clostridium Difficile Toxin A+B versus C. DIFF. QUICK CHECK Complete® Techlab as reference cell culture methods. From the results, the sensitivity was >99% and the specificity was >99%.

C. difficile TOXIN A+B - Cross reaction
Clostridium Difficile Toxin A showed consistently negative results up to 500 ng/mL with purified Toxin B.Clostridium Difficile Toxin B showed consistently negative results up to 500 ng/mL with purified Toxin A.
An evaluation was done to determine the cross reactivity of C. difficile Toxins A-BCard. There is not cross reactivity with common gastrointestinal microorganisms occasionally present in feces: *Helycobacter pylori, Campylobacter spp, E.ColiO157:H7, Listeria monocytogenes, Salmonella spp, Shigella spp, Staphilococcus aureus,Yersinia spp and Yersinia enterocolitica*

X. LIMITATIONS
- The Clostridium Difficile Toxin A+B Card is specifically designed to detect Toxin A or Toxin B antigen in the stool samples. As for any *in vitro* diagnostic procedure, the physician should confirm the test results with other clinical methods.
- A negative result does not generally exclude a *C.difficile* infection. It can be caused by proteolytic digestion of the toxins due to improper specimen storage. If a reasonable suspicion of an infection exists, another stool specimen should be investigated.
- The presence of blood in the feces samples in significant quantity may lead to false positive results in limited cases.
- A positive result does not exclude the presence of other pathogens.
- Test and control lines colours may slightly change depending on the stool sample aspect. For example dark green lines (instead of pink lines) have been reported when assaying greenish or darkish stool samples. This stool coloration appears in case of treatment of iron deficiency with ferrous fumarate. The test result should be interpreted as usual, i.e. two lines for a positive result and one line for a negative result.
- The test must be carried out within 2 hours of opening the sealed bag.

XI. BIBLIOGRAPHY
See Italian version

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		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

CONTENT (25 tests) **Ref. VC 194055**

C. DIFFICILE TOXIN A+B Card	25 Devices
Liq.Extr tubes/Extr.Solution	25 x 1.0 mL
Instruction for use	1 item

EDMA CODE 1501900200 CE IVD