CoproStrip™ C. difficile

GDH + Toxin A + Toxin B

A rapid, one step test for the simultaneous qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B antigens in human faeces.

Instruction Manual

Test kit for 20 determinations
(Catalog No.41220)

For professional in vitro diagnostic use only
Store at 2-30°C. **Do Not Freeze**

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Intended Use: The CoproStrip™ C. difficile GDH + Toxin A + Toxin B is a rapid chromatographic immunoassay combo card for the simultaneous qualitative detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human faeces that aids in the diagnosis of *C.difficile* infection.

SUMMARY AND EXPLANATION:

The gram-positive anaerobic bacillus *Clostridium difficile* is the leading causative agent of antibiotic-associated diarrhea and pseudomembranous colitiss .This pathogen is capable of causing disease that could be severe or fatal if not diagnosed on time and treated. Exposure to antibiotics is the major risk factor for *C. difficile* infection. Infection can develop if the normal gastrointestinal flora is disrupted by antibiotic therapy and a person acquires toxin-producing *C. difficile*, typically via the fecal-oral route .*C. difficile*'s key virulence factors are toxin A and toxin B. These toxins show high sequence and functional homology. Toxin A has been described as a tissue damaging enterotoxin which attracts neutrophils and monocytes and toxin B as a potent cytotoxin that degrades the colonic epithelial cells. Most virulent strains produce both toxins, however, toxin A negative/toxin B positive strains are also capable of causing disease . *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.

PRINCIPLE OF THE PROCEDURE

The CoproStrip™ C. difficile GDH + Toxin A + Toxin B is a qualitative immunoassay for detection of *Clostridium difficile* Glutamate Dehydrogenase (GDH), Toxin A and Toxin B in human fecal specimens.
The membrane of the Test A is pre-coated with monoclonal antibodies against of *Clostridium difficile* (GDH) antigen, the membrane of the Test B is pre-coated with monoclonal antibodies against Toxin A of *Clostridium difficile* antigens and the membrane of the Test C is pre-coated with monoclonal antibodies against Toxin B of *Clostridium difficile* antigens on the test lines region. During testing, the sample reacts with the red colored particles coated with anti-GDH antibodies in the Test A and/or with anti-Toxin A antibodies in the Test B and/ or with anti-Toxin B antibodies in the Test C, which were pre-dried on the test strips. The mixture moves upward on the membrane by capillary action. In the case of a positive result in the Test A the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. In the case of a positive result in the Test B the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. In the case of a positive result in the Test C the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. The mixture continues to move across the membrane to the immobilized antibody places in the control band region. A green colored band always appears in the control lines and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

MATERIALS PROVIDED

- CoproStrip™ C. difficile GDH+ Toxin A+ Toxin B devices
- Instructions for use
- Sample collection vial with buffer

MATERIALS NOT PROVIDED

- Specimen collection container
- Disposable gloves
- Timer

WARNING AND PRECAUTIONS

- For professional in vitro diagnostic use only.
- Read the instructions for use carefully before use the kit.
- Do not use after expiration date.
- Do not use product 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.
- Follow 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.

- 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.
- The test should be discarded in a proper biohazard container after testing. These containers should be discarded in accordance with local or national laws and regulations.
- A new test must be used for each sample 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), CoproStrip™ C. difficile GDH + Toxin A + Toxin B Device 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 do not mean failure on test functionality.
- Visual interpretation of the results must be done by professional user without problems in colour interpretation.

STORAGE AND STABILITY

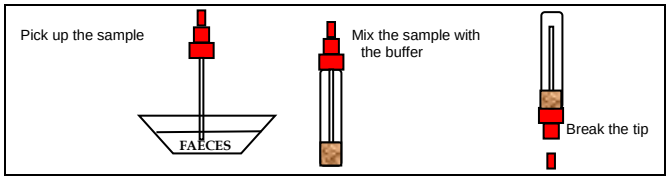
Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

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) for maximum 24 hours 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 and mix as thoroughly as possible before testing.

To process the collected stool samples (see illustration 1):
Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick into the faecal specimen to pick up the sample (approx. 50 mg).
Not to exceed the stick`s screw to avoid wrong results.
Close the vial with the buffer and stool sample. Vortex the vial for 15 seconds in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 125uL into the specimen collection vial with buffer.

Illustration 1



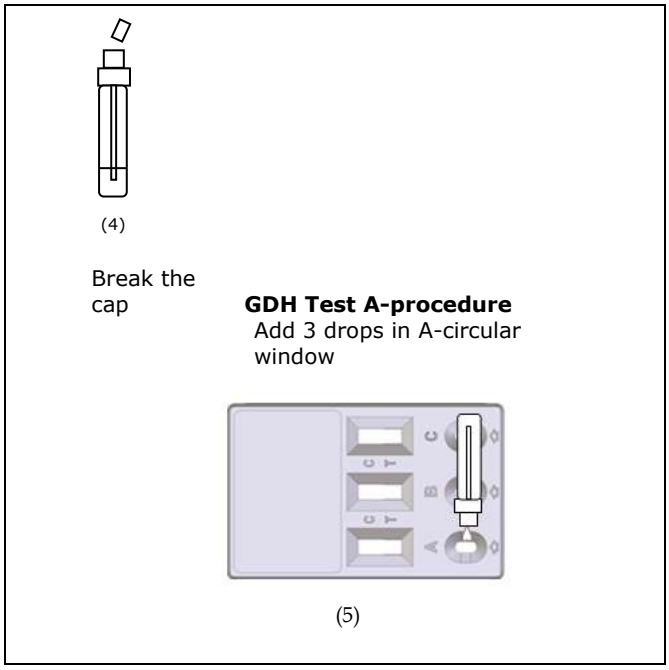
PROCEDURE

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

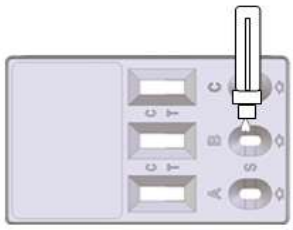
1. Remove the Device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial. (4)
3. Use a separate device for each sample. Dispense exactly 3 drops in the circular window marked with the letter A (5), 3 drops, using the same tube, in the circular window marked with the letter B (6) and 3 drops, using the same tube, in the circular window marked with the letter C (7). Start the timer.
4. Read the results at **10 minutes** after dispensing the sample.

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

Illustration 2



Toxin A Test B-procedure
Add 3 drops in B-circular window



(6)

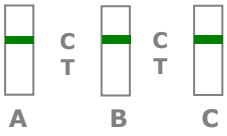
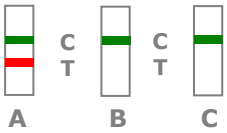
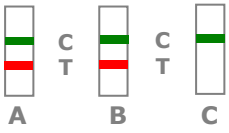
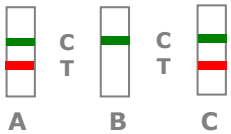
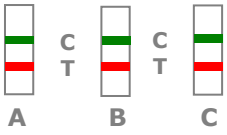
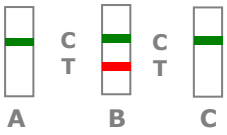
Toxin B Test C-procedure
Add 3 drops in C-circular window

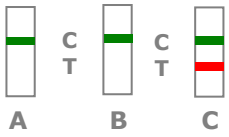
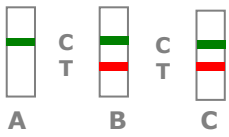
(7)

INTERPRETATION OF RESULTS

Note: The test red line intensity may vary between the different test strips. Appearance of any intensity of the red line indicates a positive result

Illustration 3

	Indicated Result	Interpretation
1.		GDH, Toxin A and Toxin B of *are* negative. No *C. difficile* infection.
2.		GDH positive. Toxin A and Toxin B are negative. *Indication of C. difficile* infection.
3.		GDH and Toxin A are positive. Toxin B is negative. *Indication of C. difficile infection. Further analysis of the sample by CE/FDA cleared assay is recommended.*
4.		GDH and Toxin B are positive. Toxin A is negative. *C. difficile* infection. *Indication of C. difficile* infection.
5.		GDH, Toxin A and Toxin B are positive. *Indication of C. difficile* infection.
6.		GDH and Toxin B are negative. Toxin A is positive. Repeat the test using a fresh sample. If result is again positive for Toxin A and negative for GDH, further analysis of the sample by CE/FDA cleared assay is recommended

7.		GDH and Toxin A are negative. Toxin B is positive. Repeat the test using a fresh sample. If results are again positive for Toxin B and negative for GDH, further analysis of the sample by CE/FDA cleared assay is recommended
8.		Toxin A and Toxin B are positive. GDH is negative. Repeat the test using a fresh sample. If results are again positive for Toxin B and negative for GDH, further analysis of the sample by CE/FDA cleared assay is recommended
9.	Any other result	Invalid result: either A, B or C, repeat the test using a fresh sample. If results remain the same further analysis of the sample by CE/FDA cleared assay is recommended

INVALID: Total absence of the green control band in one, two or the three Tests (A/B/C) regardless the appearance or not of the red test lines in one or both Tests (A/B/C). Notes: 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 using a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor. See illustration 3 above.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured band in the result line 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.

QUALITY CONTROL

Internal procedural controls are included in the test in the form of green lines appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS OF THE PROCEDURE

1. The CoproStrip™ C. difficile GDH + Toxin A+ Toxin B test will only indicate the presence of GDH, Toxin A and Toxin B of Clostridium difficile in the specimen (qualitative detection) and should be used for the detection of GDH, Toxin A and Toxin B of Clostridium difficile in stool samples; the use of other samples has not been established. Neither the quantitative value nor the rate of increase in GDH, Toxin A and Toxin B of Clostridium difficile concentration can be determined by this test.
2. The test must be carried out within 2 hours after opening the sealed bag.
3. An excess of sample could cause wrong results (brown lines appear). Dilute the sample with the buffer and repeat the test.
4. The intensity of the test line may vary from very strong at high antigens concentration to faint when antigens concentrations is close to the detection limit value of the test.
5. 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.
6. Positive results determine the presence of GDH, Toxin A and/or 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.
7. A negative result is not meaningful because of it is possible the antigens concentration in the stool samples is lower than the detection limit value. If clinical symptoms persist, a Clostridium difficile determination should be carried out on a sample from an enrichment culture.
8. 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.

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.

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT

The detection limit is 0.39 ng/mL of Clostridium difficile antigen GDH

The detection limit is 2 ng/mL of Clostridium difficile Toxin A.

The detection limit is 3.12 ng/mL of Clostridium difficile Toxin B.

SENSITIVITY AND SPECIFICITY (TEST A) GDH LINE

An evaluation was performed using the CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device vs other commercial lateral flow tests.

Discrepant samples were tested by a commercial qPCR test. (VIASURE Clostridium difficile Real Time Detection Kit, CerTest Biotec) to determine the identity of the sample.

SENSITIVITY AND SPECIFICITY (TEST A) GDH

	Evaluation Criteria			
IC test: CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device (GDH)		+	-	Total
	+	86	1	87
	-	3	160	163
	Total	89	161	250

	CoproStrip™ C. difficile GDH + Toxin A+ Toxin B (GDH) Device vs Evaluation Criteria	
		95% CI (Confidence interval)
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%

SENSITIVITY AND SPECIFICITY (TEST B AND C) TOXIN A/B

An evaluation was performed using the CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device vs other commercial lateral flow tests.

Discrepant samples were tested by a commercial qPCR test. (VIASURE Clostridium difficile Real Time Detection Kit, CerTest Biotec) to determine the identity of the sample.

	Evaluation Criteria			
IC test: CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device (Toxin A)		+	-	Total
	+	51	0	51
	-	1	198	199
	Total	52	198	250

	CoproStrip™ C. difficile GDH + Toxin A+ Toxin B (Toxin A) Device vs Evaluation Criteria	
		95% CI (Confidence interval)
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%

	Evaluation Criteria			
IC test: CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device (Toxin B)		+	-	Total
	+	40	0	40
	-	4	206	210
	Total	44	206	250

	CoproStrip™ C. difficile GDH + Toxin A+ Toxin B (Toxin B) Device vs Evaluation Criteria	
		95% CI (Confidence interval)
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%

CROSS-REACTIVITY

An evaluation was performed to determine the cross reactivity of CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device. There is no cross reactivity with common intestinal pathogens, other organisms, substances and/or fecal markers occasionally present in feces.

FOR TEST A: GDH

Adenovirus	*Coronavirus*	*Norovirus GI/Norovirus GII*
Astrovirus	*Cryptosporidium parvum*	*Peptostreptococcus anaerobius*
Campylobacter coli/jejuni	*Entamoeba histolytica*	*Respiratory Syncytial Virus*
Clostridium difficile Toxin A/Toxin B	*Escherichia coli O:111; O:026; O157:H7*	*Rotavirus*
Clostridium perfringens	*Giardia lamblia*	*Salmonella enteritidis/paratyphi A/typhi/typhimurium*
Clostridium bifermentans	*Helicobacter pylori*	*Shigella boydii/dysenteriae/ flexneri/ sonnei*
Clostridium Butyricum	*Hemoglobin (bovine and pig)*	*Staphylococcus aureus*

Clostridium Haemolyticum	*Influenza A and B*	*Streptococcus pneumococcal*
Clostridium Novyi	*Lactoferrin (bovine)*	*Streptococcus pyogenes*
Clostridium Tetani	*Legionella pneumophila*	*Transferrin (bovine)*
Clostridium Septicum	*Listeria monocytogenes*	*Yersinia enterocolitica O:3/O:9*

A specificity assay was performed for CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device (test A). This test could 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 CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device (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	*Escherichia coli O:111; O:026; O157:H7*	*Norovirus GI/Norovirus GII*
Astrovirus	*Giardia lamblia*	*Respiratory Syncytial Virus*
Campylobacter coli/jejuni	*Helicobacter pylori*	*Rotavirus*
Clostridium difficile GDH/Toxin B	*Hemoglobin (bovine and pig)*	*Salmonella enteritidis/paratyphi A/typhi/typhimurium*
Clostridium perfringens	*Influenza A and B*	*Shigella boydii/dysenteriae/ flexneri/ sonnei*
Coronavirus	*Lactoferrin (bovine)*	*Staphylococcus aureus*
Cryptosporidium parvum	*Legionella pneumophila*	*Streptococcus pneumococcal*
Entamoeba histolytica	*Listeria monocytogenes*	*Streptococcus pyogenes*
Entamoeba dispar	*Norovirus GI/Norovirus GII*	*Yersinia enterocolitica O:3/O:9*

FOR TEST C: TOXIN B

Adenovirus	*Escherichia coli O:111; O:026; O157:H7*	*Norovirus GI/Norovirus GII*
Astrovirus	*Giardia lamblia*	*Respiratory Syncytial Virus*
Campylobacter coli/jejuni	*Helicobacter pylori*	*Rotavirus*
Clostridium difficile GDH/Toxin A	*Hemoglobin (bovine and pig)*	*Salmonella enteritidis/paratyphi A/typhi/typhimurium*
Clostridium perfringens	*Influenza A and B*	*Shigella boydii/dysenteriae/ flexneri/ sonnei*
Coronavirus	*Lactoferrin (bovine)*	*Staphylococcus aureus*
Cryptosporidium parvum	*Legionella pneumophila*	*Streptococcus pneumococcal*
Entamoeba histolytica	*Listeria monocytogenes*	*Streptococcus pyogenes*
Entamoeba dispar	*Norovirus GI/Norovirus GII*	*Yersinia enterocolitica O:3/O:9*

INTERFERENCES
It was performed an evaluation to determine the possible interferences of CoproStrip™ C. difficile GDH + Toxin A+ Toxin B Device. There are no interferences against the substances and/or fecal markers occasionally present in faeces.

Exogenous interferences			
Metronidazole	Ibuprofen (*Espidifen*)	Almagato (*Almax*)	Amoxicillin
Ampicillin	Paracetamol (*Dolocatil*)	Fosfamycin (*Monurol*)	Mercaptopurine
Oseltamivir	Metamizole (*Nolotil*)	Aceltylcysteine (*Fluimucil*)	Biotine
Amantadine	Prednisone	Dexketoprofen trometamol (*Enantyum*)	Sore Throat Phenol spray
Ribavirin	Omeprazole	Levofloxacin	Tobramycin
Codeine (*Toseina*)	Naso GEL	Ciprofloxacin	Mupirocin
Benzocaine (*Angileptol*)	CVS Nasal Spray *(Cromolyn)*	Rifampicin (*Rifaldin*)	Fluticasone Propionate
Cloperastine (*Flutox*)	Afrin *(Oxymetazoline)*	Phenoxymethylpenicillin potassium	Amoxicillin

Carbocisteine (*Iniston mucolítico*)	CVS Nasal Drops *(Phenylephrine)*	Ambroxol hydrochloride (*Mucosan*)	Mercaptopurine
Loratadine	ZICAM	Macrogol 3350 (*Movicol*)	Biotine
Dexchlorpheniramine (*Polaramine*)	Phenylpropanolamine	Lysine Carbocysteinate (*Pectox*)	Sore Throat Phenol spray
Ebastine (*Ebastel*)	Loperamide hydrochloride (*Fortasec*)	Hydroxyzine dihydrochloride	Homeopathic
Acetyl Salicylic (*Adiro*)	Heparin *(Hibor)*	Lorazepam	
Endogenous interferences			
Human haemoglbin	Human transferrin	Human calprotectin	Human lactoferrin
Mucine	Human blood	Human lactoferrin	

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.

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Symbols for IVD components and Reagents			
	Manufacturer	IVD	For *in vitro* diagnostic use only
EC REP	Authorized representative		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by
DIL	Sample diluent		Only one use