

Clostridium difficile GDH+ Toxin A +Toxin B Combo Rapid Test Cassette (Feces)

For in vitro professional use only. human feces samples. rapid diagnostic test for the detection of Clostridium difficile GDH, Toxin A and Toxin B antigens

[INTENDED USE]

The BIOZEK Clostriulum difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of Clostridium difficile GDH, Toxin A and Toxin B antigens in the human feces specimen. antigens in the human feces specimen

Costrictum difficile is an anaerobic bacteria acting as an opportunistic pathogen; it grows in the intestine when the normal flora has been altered by treatment with antibiotics. ^{1,23} Toxinogenic strains of Clostrictum difficile cause infections from mild-charmea to pseudomembranous colitis,

potentially leading to death.

Disease is caused by two toxins produced by toxinogenic strains of C.difficile. Toxin A (tissue-clamaging entercloxin) and Toxin B (cytoloxin). Some strains produce both toxins A and B, some others produce Toxin B only. The potential role of a third (binary) toxin in pathogenicity is still debated.

GDH, Toxin A and Toxin B specific to C, difficile in fecal specimen.

[PRINCIPLE] The use of Glutamate Dehydrogenase (GDH) as an antigen marker of C.difficile proliferation has been shown to be very effective because all strains produce high amount of this enzyme. ^{5,6} Clostratum difficile, GDH+ Toxin A + Toxin B Combo Rapid Test Cassette allows the detection of

Clostridium difficile Rapid Test Cassette detects three distinct antigens in fecal specimens for C. difficile, viz. GDH. Toxin A and Toxin B on three different test strips in a single test cassette, thus simultaneously detecting three antigens specific Clostridium difficile.

For C. difficile-specific GDH Testing

The membrane is precoated with anti-C.diff GDH antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-C.diff GDH antibody. The mixture migrates pward on the membrane and generate a colored line.

For C. difficile-specific Toxin A Testing.

The membrane is precoated with anti-C.dfff. Toxin A antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-C.dfff Toxin A antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.dfff Toxin A antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

For C. difficile-specific Toxin B Testing

The membrane is precoated with anti-C.dfff Toxin B antibody on the test line region. During testing, the specimen reacts with the particle coated with anti-C.dff Toxin B antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.dff Toxin B antibody on the membrane chromatographically by capillary action to react with anti-C.dff Toxin B antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

To serve as a procedural control, a colored line will always appear in the control line region of all the three test strips, indicating that the proper volume of specimen has been added and membrane

[REAGENTS]

The test cassette contains anti-Clostridium difficile GDH, anti-Clostridium difficile Toxin A and anti-Clostridium difficile Toxin B antibody coated particles and anti-Clostridium difficile Toxin A and anti-Clostridium difficile Toxin B antibody coated on the

[PRECAUTIONS]

The test should remain in the sealed pouch until use.

Do not eat, drink or smoke in the area where the specimens or kits are handled.
 Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
 Wear protective clothing such as laboratory coats, disposable gloves and eye protection when

specimens are assayed.

The used test should be discarded according to local regulations.

Hundity and temperature can adversely affect results.

ISTORAGE AND STABILITY

Store as packaged at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The lest must remain in the sealed pouch until use.

DO NOT FREEZE. Do not use beyond the expiration date

ISPECIMEN COLLECTION AND PREPARATION

The stool specimens must be tested as soon as possible after collection. If necessary, original feces specimen could be stored at 2-8°C for 3 days or -20°C for longer periods of time, extracted specimen in buffer could be stored at 2-8°C for 1 week or -20°C for longer periods of time.

Make sure that the specimens are not treated with solutions containing formaldehyde or its

[MATERIAL]

Droppers Test Cassettes

 Package Insert
 Sample Extraction Buffer
 Sample Extraction Buffer
 Materials Required But Not Provided
 Timer
 Centrifuge Materials Provided

Work Station

Allow the test, specimen, Stool Containers collection buffer and/or control to equilibrate to room temperature

 To collect fecal specimens: Collect sufficient quantity of faces (1-2mL or 1-2g) in a clean, dry specimen collection container to obtain enough antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long term storage, specimens should be kept below -20°C.

) process fecal specimens:

For <u>Solid Specimens</u>:

For <u>solid Specimens</u>:

Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the fecal specimen at least 3 different sites to collect collection applicator into the fecal specimen at least 3 different sites to collect approximately 50 mg of fecas (equivalent to 1/4 of a pea). Do not scoop the fecal approximately 50 mg of fecas (equivalent to 1/4 of a pea).

specimen.
For Liquid Specimens

Hold the dropper vertically, aspirate fecal specimens, and then transfer **2 drops of the liquid specimen** (approximately 80 µL) into the specimen collection tube containing the

reaction for 2 minutes, Tighten the cap onto the specimen collection tube, and then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Leave the collection tube for

pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the full pouch. Bring the test to room temperature before opening it. Remove the test cassette from the ₫.

Hold the specimen collection tube upright and unscrew the tip of the specimen collection tube. Invert the specimen collection tube and transfer 3 full drops of the extracted specimen (approximately 120 µL) to each of the specimen well(S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.

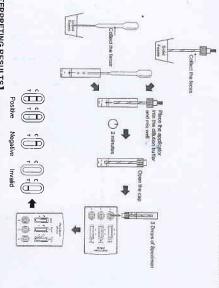
Read the results at 10 minutes after dispensing the specimen. Do not read results after

O

20 minutes.

20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the diluted sample contained in the extraction buffer vial. Collect 120µL of supernatant, dispense into the specimen well (S). Start the timer and continue from step 5 onwards in the above instructions for use.



[INTERPRETING RESULTS]

The test results appear in three different test windows respectively for GDH, Toxin A or Toxin B. The interpretation criteria remain the same for positivity or negativity for specific entigens under tests as per indication of the respective Test window. The results are to be interpreted as follows: POSITVE: Two distinct colored lines appear. One colored line should be in the control line region (C) and another appearent colored line should be in the test line region (T) another operation of the concentration of Clostrictum difficile antigens present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.

line region (1).

Illure region (1).

Illure region (1).

InvALID: Control line (c) fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact NEGATIVE. One colored line appears in the control line region (C). No line appears in the test

Vour local distributor.

[QUALITY CONTROL]

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify An internal procedural control is included in the test. A colored line

performance.

The BIOZEK Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces) is

for in vitro diagnostic use only.

2. The test is qualitative and cannot predict the quantity of antigens present in the sample. C presentation and other test results must be taken into consideration to establish diagnosis. A positive test does not rule out the possibility that other pathogens may be present.**[EXPECT VALUE]** Clinical

In a healthy individual's fecal specimens, Clostridium difficile test should give negative test result for any of the antigens tested. The Clostridium difficile GDH+Towin A+Towin B Combo Rapid Test Cassette (Fecas) has been compared with another leading commercial rapid test. The correlation between two systems is 95.3% for C.diff GDH and 96.0% for C.diff Towin A and 94.7% for Towin B. [PERFORMANCE]

Detection Limit

Detection limit values of Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette was 1ng/ml for GDH, 2ng/ml for Toxin A and 7ng/ml for Toxin B.

Clostridium difficile GDH Results Sensitivity - Specificity

3IOZEK Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette Results Positive 0 116 Other Rapid Test Negative 170 *Confidence Intervals Total Results 176 300

Relative Sensitivity: 95.1% (95%CI:*89.6%-98.2%) Relative Specificity: 95.5% (95%CI:*91.3%-98.0%) Relative Accuracy: 95.3% (95%CI:*92.3%-97.4%)

Clostridium difficile Toxin A Results

BIOZEK Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid est Cassette (Feces Results Positive 115 Other Rapid Test Negative 173 **Total Results** 180

Relative Sensitivity. 97.3% (95%Cl*88.5%-97.7%) Relative Specificity. 97.2% (95%Cl*93.6%-99.1%) Relative Accuracy. 98.0% (95%Cl*93.4%-97.9%) Ciostridium difficile Toxin B Results

Confidence

Relative Sensitivity: 91.8% (95%CI:*85.4%-96.0%)	Total Results	BIOZEK Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces)			Method
Relative Sensitivity: 91.8% (95%CI:*85.4%-96.0%)		Negative	Positive	Results	
-96.0%)	122	10	112	Positive	Other R
	178	172	6	Negative	Other Rapid Test
"Confidence intervals	300	182	118	lotal Results	

Relative Accuracy: 94.7% (95%CI:*91.5%-96.9%)

Precision

To check intra-batch accuracy (repealability), the same positive samples and a buffer solution were processed 3 times on test kits of the same batch number in the same experimental conditions. All observed results were confirmed as expected.

To check inter-batch accuracy (reproducibility), same samples (positive and buffer) were processed on test kits from three different batches. All results were confirmed as expected. Cross Reactivity

gastrointestinal pathogens occasionally present as following: Campylobacter coll Salmonella enteritidis An evaluation was performed to determine the cross reactivity of BIOZEK Clostridium difficite GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces). No cross reactivity against Listeria monocytogenes Shigella boydii Salmonella paratyphi Salmonella typhi Salmonella lyphimurium Interfering Substances Shigella dysenteriae Shigella flexneri Staphylococcus aureus Shigella sonnei rersinia enterocolítica against

E.coli 0157:H7

The following potentially Interfering Substances were added to Clostridium difficile GDH+Toxin A+Toxin B negative and positive specimens.

Oxalic acid: 60mg/dl Oxalic acid: 60mg/dl Bilirubin: 100mg/dl Urea: 2000mg/dl Urea: 2000mg/dl Glucase: 2000mg/dl [BIBLIOGRAPHIC REFERENCES] Caffeine: 40mg/dl Bilirubin: 100mg/dl Urea: 2000mg/dl Albumin: 2000mg/dl

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INDEX OF SYMBOLS 66(4) p. 175-179, 2009.

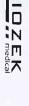
Willis DH. And JA Kraft: Confirmation that the latex-reactive protein of Clostridium difficile isa Glutamate Dehydrogenase. Journal of clinical microbiology, 30, p. 1363-1364, May 1992

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Do not use If package is damaged

Do not re-use

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