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Rapid Detection of Clostridium difficile Infection: Performance Evaluation of the GDH + Toxin A + Toxin B Combo Rapid Test Cassette

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Abstract

Clostridium difficile, an anaerobic bacterium, opportunistically thrives in the intestinal tract when the normal microbiota is disrupted by antibiotic treatment. It acts as a pathogen, capable of causing various infections ranging from mild diarrhea to life-threatening pseudomembranous colitis. The pathogenicity of this disease is attributed to two toxins: Toxin A, an enterotoxin causing tissue damage, and Toxin B, a cytotoxic. While some strains produce both toxins, others produce only Toxin B. The role of a third toxin, known as the binary toxin, in the pathogenicity of Clostridium difficile is still a subject of debate. The detection of Glutamate Dehydrogenase (GDH), an antigen marker for C. difficile proliferation, has proven to be highly effective as all strains produce this enzyme abundantly.

The Clostridium difficile GDH + Toxin A + Toxin B Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay designed to qualitatively detect the presence of Clostridium difficile GDH, Toxin A, and Toxin B antigens in human feces specimens. This study aims to assess its performance by comparing it to other high-quality products in the Point-of-Care Testing (POCT) industry. The findings underscore its significance in facilitating early diagnosis, enabling timely intervention, and preventing the spread of Clostridium difficile infection (CDI).

Keywords: Clostridium difficile; Rapid test; Chromatographic immunoassay

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Introduction

Clostridium difficile infection (CDI)

Clostridioides difficile infection (CDI or C-diff), also known as Clostridium difficile infection, is a symptomatic infection due to the spore-forming bacterium Clostridioides difficile [1]. Symptoms include watery diarrhea, fever, nausea, and abdominal pain. CDI constitutes approximately 20% of cases of antibiotic-associated diarrhea. Antibiotics can lead to detrimental changes in gut microbiota, notably reducing short-chain fatty acid absorption, resulting in osmotic or watery diarrhea [2]. Complications may include pseudomembranous colitis, toxic mega colon, colon perforation, and sepsis.

The manifestations of CDI range from mild diarrhea to severe, life-threatening inflammation of the colon [3]. In adults, clinical prediction models have identified significant diarrhea, recent antibiotic exposure, abdominal pain, fever, and a distinctive foul odor resembling horse manure as key signs of CDI. Among children, a prevalent symptom is watery diarrhea, involving at least three daily bowel movements for two or more days, often accompanied by fever, loss of appetite, nausea, and/or abdominal pain [4]. Severe infections may also lead to pronounced colon inflammation with little or no diarrhea.

Diagnosis

Before tests for detecting C. difficile toxins became available, diagnosis often relied on colonoscopy or sigmoidoscopy. While these procedures are still in use, stool testing for C. difficile toxins has become the preferred initial diagnostic method. A common approach for antigen detection is the enzyme immunoassay (EIA), which depends on the specific binding reaction between antibodies and C. difficile antigens. During the test, a fecal sample is mixed with a reagent contain specific antibodies. The presence of C. difficile antigens in the sample cause a color change and generate an optical signal.

Cytotoxicity assay

C. difficile toxins inflict damage on cultured cells. The gold standard for evaluating new CDI diagnostic techniques involves

observing and neutralizing harmful effects using specific antisera. Toxigenic culture, where organisms are cultured on selective media and tested for toxin production, remains the most accurate and reliable method, offering high sensitivity and specificity. However, this process is time-consuming and labor-intensive.

Polymerase chain reaction (PCR)

PCR (polymerase chain reaction) is a highly sensitive and specific approach for diagnosing CDI. PCR tests detect the bacterial genetic material in stool samples, providing rapid and accurate results. While PCR is valuable in diagnosing CDI, it's recommended to use it in combination with other diagnostic methods and clinical judgment to ensure accurate diagnosis and appropriate treatment. Preventive measures must also be implemented to curb CDI transmission.

Prevention

Antibiotics

Prudent antibiotic use is the most effective means of preventing CDI. In hospital settings, where CDI is common, a significant proportion of cases are linked to antibiotic use. However, despite guidelines for responsible antibiotic use, around 50% of antibiotic prescriptions are considered inappropriate in various settings. Studies demonstrate that reducing antibiotic use, both during outbreaks and in non-outbreak scenarios, is a successful strategy for decreasing CDI occurrence.

Probiotics

Some evidence indicates that probiotics may help prevent initial infection and recurrence. The use of Saccharomyces boulardii treatment for C. difficile infection in non-immunocompromised individuals might also offer benefits [5]. However, subsequent reviews haven't identified significant adverse effects linked to this treatment. Overall, probiotic use appears be safe and moderately effective in preventing Clostridium difficile-associated diarrhea [6].

Infection control

Rigorous infection control protocols are essential to minimize transmission risk. Measures like glove usage and exclusive use of noncritical medical devices for CDI patients effectively limit C. difficile spread within hospital environments. These precautions should persist for at least 2 days after diarrhea cessation in hospitalized CDI patients to minimize transmission risk.

Evaluation of AllTest *Clostridium difficile* GDH + Toxin A +Toxin B Combo Rapid Test

Objective

The primary objective of this evaluation report is to assess the reliability and performance of the Clostridium difficile GDH + Toxin A + Toxin B Combo Rapid Test cassette (feces) for the rapid diagnosis of Clostridium difficile GDH, Toxin A, and Toxin B antigens. The aim is to provide a reliable, rapid, and accurate tool for the timely diagnosis of Clostridium difficile GDH, Toxin A, and Toxin B.

Method

Conduct an in vitro diagnostic test using the rapid test device to detect Clostridium difficile GDH, Toxin A, and Toxin B antigens in human feces specimens and compare with other high-quality rapid tests to validate of the performance.

Materials

The materials provided for the Clostridium difficile GDH + Toxin A +Toxin B Combo Rapid Test include test cassettes, package insert, droppers and specimen collection tube with buffer.

Specimen collection and preparation

Ensure that the test, specimen, and collection buffer have reached room temperature (15-30°C) prior to conducting the test.

Collection of fecal specimens

Collect an adequate amount of feces (1-2mL or 1-2g) in a clean, dry specimen collection container to obtain sufficient antigens. For optimal results, perform the assay within 6 hours of collection. If the specimen cannot be tested within 6 hours, it can be stored at 2-8°C for up to 3 days. For extended storage, maintain specimens below -20°C.

Processing of fecal specimens

For solid specimens

Open the cap of the specimen collection tube and gently insert the specimen collection applicator into the fecal specimen at least three different sites, collecting approximately 50 mg of feces (equivalent to 1/4 of a pea). Avoid scooping the fecal specimen.

For liquid specimens

Hold the dropper vertically, aspirate the fecal specimen, and transfer 2 drops (approximately 80 µL) into the specimen collection tube containing the extraction buffer.

Close the cap tightly and vigorously shake the specimen collection tube to mix the specimen and extraction buffer. Allow the tube to react for 2 minutes.

Ensure the test pouch attains room temperature before opening it. Remove the test cassette from the foil pouch and use it as soon as possible. For optimal results, perform the test immediately after unsealing the foil pouch.

Hold the specimen collection tube in an upright position, and carefully remove the tube tip by unscrewing it. Invert the tube to facilitate the transfer of the extracted specimen. Dispense 3 complete drops of the specimen, equivalent to approximately 120 µL, into each designated specimen well on the test cassette. Initiate the timer, being cautious to prevent of air bubble entrapment within the specimen wells. Wait for 10 minutes after dispensing the specimen before interpreting the results. Importantly, refrain from interpreting results after 20 minutes have elapsed.

Performance characteristics

The Clostridium difficile Rapid Test Cassette is capable of detecting three distinct antigens specific to C. difficile in fecal specimens:

GDH, Toxin A and Toxin B. These antigens are detected on three separate test strips housed within a single test cassette, enabling the simultaneous identification of these three antigens critical C. difficile markers.

For C. difficile-specific GDH testing

The test line region of the membrane is pre-coated with an antibody designed to target C.diff GDH. During the testing process, the specimen interacts with particles coated with an antibody that recognizes C.diff GDH. Facilitated by capillary action, the mixture ascends along the membrane. This interaction generates a colored line, visually indicating the presence of C. diff GDH in the specimen (Table 1).

For C. difficile-specific toxin a testing

The test line region of the membrane is pre-coated with an antibody designed to target C.diff Toxin A. During the testing process, the specimen interacts with particles that are coated with an antibody that recognizes C.diff Toxin A. Facilitated by capillary action, the mixture ascends along the membrane and reacts with the anti-C.diff Toxin A antibody present on the membrane, resulting in the formation of a colored line. The presence of this colored line in the test line region indicates a positive result, indicating the presence of C.diff Toxin A. Conversely, the absence of the colored line indicates a negative result, suggesting the absence of C.diff Toxin a (Table 2).

For C. difficile-specific Toxin B testing

The membrane is precoated with anti-C.diff Toxin B antibody on the test line region. During testing process, the specimen reacts with the particle coated with anti-C.diff Toxin B antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-C.diff 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 wicking has occurred (Table 3).

Summary

The comprehensive comparison experiments in Tables 1-3 with the other top-tier products in the industry, have demonstrated that the Clostridium difficile GDH + Toxin A +Toxin B Combo Rapid Test from Hangzhou AllTest Biotech Co., Ltd performed well. The test has exhibited exceptional levels of specificity, sensitivity, and accuracy.

The high standards of product quality observed in this rapid

Table 1. Clostridium difficile GDH Results.

Method		Other Rapid Test		Total Results
Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces)	**Results**	**Positive**	**Negative**	
	Positive	116	8	124
	Negative	6	170	176
Total Results		122	178	300

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

Table 2. Clostridium difficile Toxin A results.

Method		Other Rapid Test		Total Results
Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces)	**Results**	**Positive**	**Negative**	
	Positive	115	5	120
	Negative	7	173	180
Total Results		122	178	300

Relative Sensitivity: 94.3% (95%CI:*88.5%-97.7%) *Confidence Intervals
Relative Specificity: 97.2% (95%CI:*93.6%-99.1%)
Relative Accuracy: 96.0% (95%CI:*93.1%-97.9%)

Table 3. Clostridium difficile Toxin B results.

Method		Other Rapid Test		Total Results
Clostridium difficile GDH +Toxin A +Toxin B Combo Rapid Test Cassette (Feces)	**Results**	**Positive**	**Negative**	
	Positive	112	6	118
	Negative	10	172	182
Total Results		122	178	300

Relative Sensitivity: 91.8% (95%CI:*85.4%-96.0%) *Confidence Intervals
Relative Specificity: 96.6% (95%CI:*92.8%-98.8%)
Relative Accuracy: 94.7% (95%CI:*91.5%-96.9%)

test underscore its credibility as a reliable diagnostic solution. The concurrent detection of three critical markers GDH, Toxin A, and Toxin B – not only streamlines the testing process but also optimizes resources and time utilization, thereby enhancing overall testing efficiency.

The results obtained from the tested samples undeniably affirm that the AllTest Clostridium difficile GDH + Toxin A + Toxin B Combo Rapid Test satisfactorily fulfills the requirements for professional in vitro diagnostics. This test bears significant clinical importance, enabling early and precise identification of Clostridium difficile infection, thereby facilitating prompt intervention and contributing to effective infection control measures.

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