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Performance Evaluation of Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (Feces, ICD-R635B) via Clinical Validation

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Abstract: This clinical validation study comprehensively assesses the diagnostic performance of the Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (ICD-R635B, Hangzhou Alltest Biotech Co,.Ltd), an in vitro qualitative diagnostic device for simultaneous detection of Clostridium difficile glutamate dehydrogenase (GDH), Toxin A and Toxin B antigens in human fecal specimens. A commercial CERTEST Clostridium difficile GDH+Toxin A+B rapid test kit (CERTEST BIOTEC S.L.) was used as the reference assay for performance benchmarking. A total of 163 archived fecal specimens from patients with suspected Clostridium difficile infection (CDI) were enrolled, including 43 reference-positive and 120 reference-negative samples. The investigational assay yielded robust diagnostic performance: GDH relative sensitivity 97.7% (95% CI: 87.7%–99.9%), specificity 95.0% (95% CI: 89.4%–98.1%), accuracy 95.7% (95% CI: 91.4%–98.3%); Toxin A sensitivity 95.3% (95% CI: 84.2%–99.4%), specificity 97.5% (95% CI: 92.9%–99.5%), accuracy 96.9% (95% CI: 93.0%–99.0%); Toxin B sensitivity 95.3% (95% CI: 84.2%–99.4%), specificity 99.2% (95% CI: 95.4%–99.9%), accuracy 98.2% (95% CI: 94.7%–99.6%). All tests were performed within the validated operating temperature range (2-30 ℃). Test results were uniformly interpreted via the LF Reader Plus instrument for standardized, objective reading; no manual visual interpretation of test lines was performed per official product specifications, with final positive/negative/invalid results directly displayed on the instrument screen. All cassettes are single-use disposable devices. The investigational kit exhibited high concordance with the reference assay with no obvious cross-reactivity or abnormal invalid rates. This triple-biomarker rapid test provides reliable, fast point-of-care CDI detection for clinical laboratory and bedside screening.

1. Introduction

Clostridium difficile (recently reclassified as Clostridioides difficile) is an anaerobic, spore-forming opportunistic enteropathogen and the leading cause of global healthcare-associated infectious diarrhea[1]. As stated in the clinical background of the ICD-R635B clinical study report,

antibiotic treatment disrupts normal intestinal flora, enabling extensive proliferation of toxigenic C. difficile strains. These strains release cytotoxic virulence factors, causing a spectrum of CDI manifestations from mild self-limiting watery diarrhea to severe fatal complications including pseudomembranous colitis, toxic megacolon, bowel perforation and sepsis[2,3]. Two key exotoxins primarily determine CDI severity: enterotoxin Toxin A that induces intestinal inflammation and fluid secretion, and potent cytotoxin Toxin B that impairs colonic epithelial cells[4]. Some epidemic hypervirulent strains only produce Toxin B, which invalidates single-toxin detection and necessitates dual-toxin testing for accurate identification of all toxigenic isolates [5]. Certain C. difficile ribotypes encode a third binary toxin, while its independent pathogenic role in clinical settings remains controversial.

Glutamate dehydrogenase (GDH) is a constitutively and highly expressed cytoplasmic enzyme in nearly all toxigenic and non-toxigenic C. difficile strains, serving as an optimal pan-species biomarker for screening bacterial fecal carriage, as documented in the trial background [6]. International clinical guidelines from ESCMID, IDSA/SHEA and ACG advocate a two-tier CDI diagnostic algorithm: initial GDH antigen screening to exclude negative cases, followed by toxin testing to distinguish asymptomatic colonization from clinically significant toxigenic infection. NAAT detection of tcdA/tcdB toxin genes is required to confirm samples with inconsistent GDH-positive and toxin-negative results [7,8]. Single-analyte assays have prominent diagnostic defects: independent toxin immunoassays have low analytical sensitivity and high false-negative rates, while GDH-only tests fail to distinguish asymptomatic colonization from active toxin-mediated CDI [9]. Multiplex rapid combo tests that simultaneously detect GDH, Toxin A and Toxin B in a single fecal specimen address this clinical deficiency, achieving integrated screening and preliminary confirmation in a single workflow without secondary specimen processing.

Conventional CDI laboratory diagnostic methods include toxigenic culture, cell cytotoxicity neutralization assay, enzyme immunoassays (EIAs) and NAAT molecular detection [10]. As the reference standard, toxigenic culture requires days of anaerobic incubation and professional microbiology facilities, which do not support same-day clinical diagnosis. Cytotoxicity assays depend on cell culture systems with 24-48 hours of incubation. Although NAATs offer high analytical sensitivity, their application is limited in community hospitals, outpatient departments and resource-limited settings due to the requirement for expensive equipment, professional technicians and rigorous biosafety protocols. Traditional single-target EIAs need batch detection and lengthy manual operation, making them unsuitable for urgent bedside CDI screening.

The Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (Feces, Ref.: ICD-R635B, Hangzhou AllTest Biotech Co., Ltd.) is a CE-marked qualitative in vitro diagnostic medical device recorded in the product label document, designed for simultaneous detection of all three core CDI biomarkers from unformed human stool specimens. This clinical validation study aims to systematically quantify its relative sensitivity, relative specificity and overall diagnostic accuracy against a marketed reference combo rapid test kit (CERTEST C. difficile GDH+Toxin A+B) using a cohort of 163 well-characterized fecal samples from suspected CDI patients, to validate its suitability as a primary point-of-care screening tool for routine clinical CDI diagnosis.

2. Experimental Procedures

2.1 Source of Clinical Samples

A total of 163 non-duplicate unformed fecal specimens were retrospectively collected from patients with clinically suspected CDI. All samples were pre-classified by the reference CERTEST assay, including 43 positive and 120 negative specimens, covering typical high-risk CDI populations. Specimens were stored and processed in accordance with standard microbiology

protocols: frozen samples were fully thawed, equilibrated to 2-30 ℃, and thoroughly homogenized before testing, with no preservatives added to avoid matrix interference.

2.2 Test Kits and Procedures

This study adopted a parallel testing design for performance evaluation. The investigational device was the Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (ICD-R635B, Hangzhou AllTest Biotech Co., Ltd.), a CE-marked in vitro diagnostic single-use test kit. The commercially available CERTEST Clostridium difficile GDH+Toxin A+B Rapid Test (CERTEST BIOTEC S.L., Spain) was selected as the reference assay. Each fecal specimen was simultaneously detected by the investigational rapid test cassette and the reference kit under consistent ambient conditions, with all tests performed by trained laboratory personnel to eliminate operational errors.

Prior to testing, all test components and thawed fecal specimens were equilibrated to room temperature (2-30 ℃) for no less than 15 minutes. Firstly, for specimen preparation, a small amount of fecal sample was added to the matching extraction tube containing assay buffer, vigorously vortexed for 10 seconds to fully disperse stool particles and then settled for 2 minutes to obtain clarified supernatant. Secondly, the clear fecal supernatant was vertically dispensed into the sample well (S) of the test cassette with a matched dropper. Thirdly, the cassette was kept flat on a horizontal surface during capillary migration, and the timer was started immediately after sample loading. Finally, test cassettes were placed into the LF Reader Plus instrument at 10 minutes post sample loading for automatic, standardized result interpretation; any readings obtained after 20 minutes were defined as invalid, a visual reading was not taken throughout the test process.In accordance with the product's, instructions for use, the LF Reader Plus completes automatic scanning and data analysis of the cassette and directly outputs and displays final positive, negative, or invalid results on the instrument screen, eliminating all manual band interpretation steps.

3. Performance Analysis

3.1 Analysis of Core Diagnostic Performance Metrics

All comparative contingency tables stratifying GDH, Toxin A and Toxin B results obtained with the investigational cassette versus the CERTEST reference assay are summarised and displayed in Tables 1–3. Each table is supplemented with calculated relative sensitivity, relative specificity and overall diagnostic accuracy, as well as the corresponding 95% confidence intervals (95% CI).

3.1.1 GDH Biomarker Clinical Performance

Table 1: Comparative concordance – AllTest Combo GDH vs. Reference CERTEST GDH

ICD-R635B GDH Result	Reference Positive	Reference Negative	Row Total
Positive	42	6	48
Negative	1	114	115
Column Total	43	120	163

Relative Sensitivity: 97.7% (95% CI: 87.7%–99.9%)

Relative Specificity: 95.0% (95% CI: 89.4%–98.1%)

Overall Diagnostic Accuracy: 95.7% (95% CI: 91.4%–98.3%)

The GDH assay achieved excellent screening sensitivity, missing only one positive sample. The 95.0% specificity is consistent with inherent assay characteristics, as GDH positivity includes both toxigenic and non-toxigenic colonization, which matches the standard two-tier diagnostic workflow

requiring subsequent toxin confirmation.

3.1.2 Toxin A Biomarker Clinical Performance

Table 2: Comparative concordance – AllTest Combo Toxin A vs. Reference CERTEST Toxin A

ICD-R635B GDH Result	Reference Positive	Reference Negative	Row Total
Positive	41	3	44
Negative	2	117	119
Column Total	43	120	163

Relative Sensitivity: 95.3% (95% CI: 84.2%–99.4%)
Relative Specificity: 97.5% (95% CI: 92.9%–99.5%)
Overall Diagnostic Accuracy: 96.9% (95% CI: 93.0%–99.0%)

Toxin A detection exhibited balanced high sensitivity and specificity with minimal false-positive and false-negative results, effectively avoiding non-specific interference from intestinal flora and reducing misdiagnosis of toxigenic CDI.

3.1.3 Toxin B Biomarker Clinical Performance

Table 3: Comparative concordance – AllTest Combo Toxin B vs. Reference CERTEST Toxin B

ICD-R635B GDH Result	Reference Positive	Reference Negative	Row Total
Positive	41	1	42
Negative	2	119	121
Column Total	43	120	163

Relative Sensitivity: 95.3% (95% CI: 84.2%–99.4%)
Relative Specificity: 99.2% (95% CI: 95.4%–99.9%)
Overall Diagnostic Accuracy: 98.2% (95% CI: 94.7%–99.6%)

Toxin B achieved the highest specificity among the three biomarkers, which is clinically critical given that Toxin B is the dominant virulence factor of almost all pathogenic C. difficile strains, including Toxin A-deficient hypervirulent variants. Equal sensitivity to Toxin A ensures comprehensive coverage of all toxigenic isolates.

3.2 Discussion

3.2.1 Clinical Performance Interpretation

The ICD-R635B assay demonstrated excellent and balanced diagnostic performance across all three biomarkers, meeting international CDI screening requirements. The high GDH sensitivity ensures reliable exclusion of negative cases, while moderate specificity conforms to universal GDH assay characteristics that require reflex toxin testing. The high specificity of Toxin A and particularly Toxin B minimizes false-positive results, avoiding unnecessary antibiotic treatment and patient isolation. The triple-marker integrated design compensates for the defects of single-target assays, streamlining laboratory workflows and shortening detection turnaround time. The narrow 95% CIs verified stable and statistically credible test results.

3.2.2 Operational Advantages

Compared with NAAT, cytotoxicity assays and traditional EIAs, the ICD-R635B cassette features low equipment dependence and simple operation. The supporting LF Reader Plus instrumental reading system eliminates subjective visual judgment errors of traditional rapid tests.

Full-process automatic scanning and direct screen output realize standardized, traceable and highly consistent results. The 10-minute rapid detection and wide 2-30 ℃ temperature adaptability support point-of-care application in resource-limited settings. Single-use disposable design also reduces cross-contamination risks.

3.2.3 Study Limitations

This assay strictly depends on LF Reader Plus for result interpretation and does not support visual read per official specifications. As a qualitative immunoassay, it does not quantify antigen concentration of the antigen to assess the severity of the disease. Like all immunoassays, false-negative results can be obtained in specimens with extremely low antigen levels. In addition, this study adopted a commercial rapid test as the reference rather than gold-standard toxigenic culture or NAAT. All samples were collected from a single laboratory, and further multicenter verification with gold-standard reference methods is required. The assay is only for use with unformed human fecal specimens only.

3.2.4 Comparison with Other CDI Modalities

While NAAT offers superior analytical sensitivity, it requires sophisticated equipment and professional molecular personnel, limiting its routine screening application. Compared with visual-read rapid kits and single-target EIAs, the LF Reader Plus-based triple-marker assay provides more accurate and consistent results and completes screening and confirmation in one workflow. In contrast to time-consuming toxigenic culture, it delivers real-time actionable results to guide immediate clinical management and infection control.

4. Conclusion

The Clostridium difficile GDH+Toxin A+Toxin B Combo Rapid Test Cassette (ICD-R635B) demonstrates strong clinical diagnostic performance with fecal samples from suspected CDI patients with stable validated statistical data. The GDH test achieves 97.7% relative sensitivity and 95.0% specificity; Toxin A has 95.3% sensitivity and 97.5% specificity, and Toxin B features 95.3% sensitivity together with an excellent specificity of 99.2%. The three biomarkers deliver overall accuracy ranging from 95.7% to 98.2%, backed by narrow 95% confidence intervals. As a multiplex rapid test, it detects pan-species C. difficile GDH and pathogenic Toxins A and B within 10 minutes in one run, removing the need for independent screening and confirmation steps. It supports an operating temperature of 2–30 ℃ and is paired with LF Reader Plus for standardized instrumental reading, requiring only basic operational training rather than sophisticated expertise. Compatible with the international two-tier CDI diagnostic algorithm, ICD-R635B works as a credible frontline screening assay. Full-process instrumental scanning and direct on-screen result output standardize testing procedures, eliminate uncertainties and inconsistency caused by traditional manual visual reading, reduce reading bias, and ensure stable, repeatable results across clinical environments.

Negative GDH results effectively exclude C. difficile carriage and avoid redundant tests. For GDH-positive specimens, simultaneous toxin testing rapidly differentiates toxigenic infection from asymptomatic colonization, guiding targeted NAAT confirmation for inconsistent results. Well-balanced high sensitivity and specificity help reduce missed cases and inappropriate treatment, optimizing patient management, hospital infection control and laboratory resource allocation. Further multicenter prospective trials adopting toxigenic culture and NAAT as gold standards, alongside interference tests targeting gastrointestinal flora, antibiotics and stool contaminants, will consolidate its clinical reliability and advance its routine widespread application.

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