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Rapid and Sensitive Loop-Mediated Isothermal Amplification Test for *Clostridium difficile* Detection Challenges Cytotoxin B Cell Test and Culture as Gold Standard▽

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Compared to the composite gold standard cytotoxin B assay and toxigenic culture, the loop-mediated isothermal amplification (LAMP) test for *Clostridium difficile* had a sensitivity and specificity of 98%, positive predictive value of 92%, and negative predictive value of >99%. A one-hour turnaround time for the LAMP test provides rapid diagnosis and cost savings.

Intestinal germination of *Clostridium difficile* spores and toxin A and B production during antibiotic treatment often lead to diarrhea, currently known as *C. difficile* infection (CDI). Diagnosis is usually based on clinical history combined with several laboratory tests (1). The cell culture cytotoxin B assay (CTBA) has remained the reference standard for detection of cytotoxin-producing *C. difficile* (3, 17), often together with culture of cytotoxin-producing *C. difficile* isolates (TC) (4, 19). However, rapid toxin A and B enzyme immunoassays (EIAs), frequently used to save cost and labor, often display suboptimal sensitivity and are no longer recommended (12, 15). More sensitive glutamate dehydrogenase antigen (GDH) tests have been commonly suggested for screening, but these are often recommended with EIA, CTBA, and/or toxin B PCR for specificity reasons (6, 7, 16). Combined lateral flow tests (GDH and toxin A and B), together with random-access toxin B PCR, provide a sensitivity close to 100%, with a turnaround time of 30 to 90 min (20). Nevertheless, these algorithms include multiple-step procedures, sometimes with conflicting results and relatively high costs. As simplified stand-alone amplification testing of stools could evolve as the future diagnostic option (19), we investigated a novel rapid loop-mediated isothermal amplification (LAMP) technique (14) designed for the detection of toxigenic *C. difficile*. In this study, LAMP performance was compared to our *C. difficile* reference diagnostics, CTBA and TC.

Consecutive stool specimens (n = 272) submitted for *C. difficile* diagnostics from hospitals and communities in Örebro County, Sweden, during May and June 2010 were examined. We detected primary CDI (n = 41 specimens) as well as recurrences (n = 9 specimens) positive by the current reference method (CTBA plus TC). The median age of CDI patients was 83 years (range, 62 to 96 years), as opposed to 68 years for non-CDI patients (range, 3 months to 96 years).

Stool samples were filtered (0.45-μm pore size), and 20 μl of filtrate was distributed to McCoy cells (96-well microtiter plate) and incubated for 48 h at 37°C, 5% CO_2. Cytopathological effects on ≥50% of cells were interpreted as positive (23). All stool samples were also cultured anaerobically on cycloserine-cefoxitin-fructose agar (CCFA) for 48 h and confirmed as *C. difficile* by using a slide test (*C. difficile* test kit, Oxoid, Basingstoke, United Kingdom) (2). The isolates cultured from primarily CTBA-negative stools were incubated in chopped meat broth and tested for cytotoxicity after 12, 24, and 48 h. A positive result in the primary CTBA and/or TC was considered positive for CDI. The LAMP assay (Illumigene, Meridian Bioscience Inc., Cincinnati, OH), which targets a conserved 204-bp sequence within the *tcdA* region of the *C. difficile* pathogenicity locus (PaLoc) (18), was performed according to the manufacturer's instructions.

Of the 272 stool specimens, 50 (18.4%) were positive using the reference method, i.e., positive in CTBA and/or TC, and 49 of these were also positive using the LAMP test. The discrepant sample was positive by TC only; however, this was after isolating only a few colonies and repeated subculturing (4 days) for purity and toxin testing. Using CTBA plus TC as a gold standard for true positive specimens, the LAMP assay displayed a sensitivity and specificity of 98% and negative (NPV) and positive predictive values (PPV) of 99% and 92%, respectively (Table 1). The sensitivity and specificity of CTBA alone were 72% and 100%, respectively. An additional four specimens were positive by LAMP test only (Table 1), but only one of these could be confirmed as a true positive using an in-house PCR detecting *C. difficile rpoA* (8).

The median turnaround time for the positive specimens was 24 h (interquartile range [IQR], 24 to 72 h) for the CTBA-plus-TC standard, compared to 1 h for the LAMP test (Sign test, $P < 0.001$).

In this study, we challenged the widely accepted CTBA-plus-TC gold standard for *C. difficile* diagnostics by a novel diagnostic test (Illumigene) using LAMP technology. The sensitivity and specificity of the LAMP test were both 98%. However, four additional specimens were positive in the LAMP assay only, of which one was confirmed positive using an in-house *rpoA* PCR. When using sensitive diagnostics like LAMP, its important to test only patients with CDI features like diarrhea (≥3 loose stools/24 h) or ileus and not asymptomatic

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TABLE 1. The novel *Clostridium difficile* diagnostic assay loop-mediated isothermal amplification (LAMP; Illumigene) compared to cell culture cytotoxin B (CTBA) and/or toxigenic culture (TC) as the gold standard[a]

Diagnostic assay	Diagnostic assay compared to CTBA plus TC							
	Sensitivity		Specificity		PPV		NPV	
	% (no. of specimens/total no.)	CI (%)	% (no. of specimens/total no.)	CI (%)	% (no. of specimens/total no.)	CI (%)	% (no. of specimens/total no.)	CI (%)
LAMP	98 (49/50)	89–100	98 (218/222)	95–99	92 (49/53)	82–98	99 (218/219)	97–100
CTBA	72 (36/50)	57–84	100 (222/222)	98–100	100 (36/36)	90–100	94 (222/236)	90–97
TC	100 (50/50)	93–100	100 (222/222)	98–100	100 (50/50)	93–100	100 (222/222)	98–100

[a] One of the four specimens that tested positive by LAMP only was positive in an in-house *rpoA* PCR, indicating that both the sensitivity and specificity of the LAMP test are higher. PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

colonization from a heavily contaminated hospital environment (10, 15).

All four patients who tested positive only by LAMP tested negative for other fecal pathogens. One had typical CDI signs and was sampled twice in 24 h; the first sample was positive only by LAMP and the second by all three methods (CTBA, TC, and LAMP). Two of the patients were hospitalized with active inflammatory bowel disease and initially tested positive by LAMP only. Due to unresolved diarrhea, these two patients were positive in CTBA, TC, and LAMP 1 week and 2 weeks later, and both responded to CDI treatment. The fourth patient had a chronic leg ulcer due to *Bacteroides fragilis* and was treated with multiple courses of antibiotics 3 weeks before developing diarrhea, and both ulcer and CDI subsequently responded to metronidazole. Accordingly, all these four cases had clinical symptoms adherent to CDI, and the LAMP test may have an even higher sensitivity, i.e., might detect CTBA-plus-TC-negative specimens. Furthermore, the already high specificity (98%) of the LAMP test is comparable to that of most real-time PCRs and EIAs (9, 13, 21, 22). Considering the prevalence of *C. difficile* in the present study (18%), the PPV (92%) was also well above most EIAs, GDH tests, and real-time PCRs according to a recent review (3). The NPV was 99% and superior to that of assays such as the GDH test (86%) (7) in ruling out *C. difficile* diarrhea. Nevertheless, targeting *tcdA*, as in the LAMP test, might be suboptimal due to the importance of toxin B in virulence (11) and the existence of toxin A-negative strains (5). Further testing of toxinotypes (18) and toxin A- and B-negative strains may be needed for optimal NPV, and a positive laboratory test result should always be evaluated in the clinical context of CDI.

In conclusion, LAMP proved to be a rapid (1-hour), easily performed, standardized, and accurate test of use for any clinician diagnosing and treating CDI, potentially decreasing morbidity and nosocomial spread of *C. difficile*.

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