

Multicenter Evaluation of the Quidel Lyra Direct *C. difficile* Nucleic Acid Amplification Assay

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Clostridium difficile is a Gram-positive bacterium commonly found in health care and long-term-care facilities and is the most common cause of antibiotic-associated diarrhea. Rapid detection of this bacterium can assist physicians in implementing contact precautions and appropriate antibiotic therapy in a timely manner. The purpose of this study was to compare the clinical performance of the Quidel Lyra Direct *C. difficile* assay (Lyra assay) (Quidel, San Diego, CA) to that of a direct cell culture cytotoxicity neutralization assay (CCNA) and enhanced toxigenic culture. This study was performed at three geographically diverse laboratories within the United States using residual stool specimens submitted for routine *C. difficile* testing. Residual samples were tested using the Lyra assay on three real-time PCR platforms, and results were compared to those for direct CCNA and enhanced toxigenic culture. The test results for all platforms were consistent across all three test sites. The sensitivity and specificity of the Lyra assay on the SmartCycler II, ABI 7500 Fast DX, and ABI QuantStudio DX instruments compared to CCNA were 90.0% and 93.3%, 95.0% and 94.2%, and 93.8% and 95.0%, respectively. Compared to enhanced toxigenic culture, the sensitivity and specificity of the Lyra assay on the SmartCycler II, ABI 7500, and QuantStudio instruments were 82.1% and 96.9%, 89.3% and 98.8%, and 85.7% and 99.0%, respectively. Overall, the Lyra assay is easy to use and versatile and compares well to *C. difficile* culture methods.

Clostridium difficile is a Gram-positive, anaerobic bacillus, which has emerged as a major nosocomial pathogen and the leading infectious cause of antibiotic-associated diarrhea and pseudomembranous colitis (1). In the United States, the number of *C. difficile* infections (CDI) in hospitalized patients has increased from approximately 150,000 patients in 2001 to >300,000 patients in 2005 and continues to rise (2). The increased economic burden in hospitalized patients due to CDI has been estimated at \$9,822 to \$13,854 per patient, and total patient costs (health care costs plus lost wages) associated with CDI have been estimated to exceed \$1 billion annually in the United States alone (3, 4). Several recent studies have demonstrated that rapid and accurate detection of *C. difficile* is an important component of combating hospital-acquired CDI and can have a significant benefit to patients and hospitals from a financial and clinical perspective (4–6).

The most appropriate testing strategy for detection of *C. difficile* is not standardized and remains controversial. Several traditional (nonmolecular) techniques are currently employed in the diagnosis of *C. difficile* disease. Enzyme immunoassays (EIAs) test for the presence of either *C. difficile* cytotoxins or glutamate dehydrogenase (GDH) (a *C. difficile* metabolic enzyme). These assays can be performed within a few hours, but they lack sensitivity and specificity, and the GDH assays cannot differentiate between cytotoxic and noncytotoxic strains of *C. difficile*. EIAs are not recommended as the sole diagnostic for detection of *C. difficile* disease (1, 7–11). Cell culture cytotoxicity neutralization assays (CCNA) detect the presence of *C. difficile* cytotoxin by inoculating cell cultures with clarified stool specimens in the presence and absence of *C. difficile* antitoxins and can take up to 48 h to complete. Finally, enhanced toxigenic culture utilizes traditional *C. difficile* culture methods followed by CCNA on suspected isolates. The Infectious Disease Society of America and Society for Healthcare Epidemiology of America (IDSA/SHEA) guidelines state that

enhanced toxigenic culture is the gold standard to which all assays should be compared due to the high sensitivity and specificity, but that this type of testing is not clinically practical due to the slow turnaround time (2 to 3 days) and the lack of standardized protocols (1, 7).

Molecular diagnostics may allow laboratories to combine the best features of all traditional *C. difficile* diagnostics from the speed and ease of EIAs to the high sensitivity and specificity of enhanced toxigenic culture (12, 13). One recent study shows that the number of unnecessary days of contact precaution and unjustified antibiotic usage decreased by nearly 40% for those patients who were diagnosed as negative for CDI by molecular testing compared to those diagnosed with CCNA or enhanced cell culture. The same study showed that the use of molecular testing decreased the length of hospitalization on average by more than 7 days compared to that for CCNA or enhanced cell culture (6).

The Quidel Lyra Direct *C. difficile* assay (Lyra assay) (Quidel, San Diego, CA) is a qualitative real-time PCR assay that detects the presence of the *C. difficile* *tcdA* and/or *tcdB* gene in liquid or soft stool specimens. Specimens are processed through a simple preparation step that does not require specialized equipment. Processed specimens are tested via a standard TaqMan real-time PCR assay utilizing primers/probes that detect but do not distinguish

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the *tcdA* and *tcdB* genes. The purpose of this study was to compare the clinical performance of the Lyra assay to that of direct CCNA and enhanced toxigenic culture using residual specimens from three geographically diverse laboratories within the United States. Testing with the Lyra assay was performed using three real-time PCR platforms, and results were compared to those for direct CCNA and enhanced toxigenic culture.

MATERIALS AND METHODS

Participating centers and overall study design. Three laboratories participated in a prospective study to assess the clinical performance of the Lyra assay: The Medical College of Wisconsin, Milwaukee, WI; The Ohio State University, Columbus, OH; and Laboratory Alliance of New York, Syracuse, NY. All of the laboratories received the appropriate Institutional Review Board approvals or waivers consistent with local human subject research requirements. All sites performed the Lyra assay with residual deidentified stool specimens that were submitted for routine *C. difficile* testing according to each laboratory's standard practices. An aliquot of each stool specimen was submitted to a central reference laboratory (Diagnostic Hybrids, Athens, OH) for direct cell culture cytotoxicity neutralization assay (CCNA) and enhanced toxigenic culture (see Materials and Methods, below).

Specimen collection and handling. A minimum of 2 ml of liquid stool or a marble-sized soft stool specimen that takes the shape of its container was required for a sample to be enrolled. Each specimen was tested according to the laboratory's standard of care, and an aliquot was then transferred to a new tube with a sterile paddle. Specimens were assigned unique specimen identification numbers, and the following patient demographics were recorded: gender, age, collection date, and presence or absence of *C. difficile* by the laboratory's routine method. Each specimen was inoculated into a vial of Lyra process buffer 1 within 24 h of collection. The remaining specimen aliquots were stored and shipped to the reference laboratory at 2 to 8°C, where reference testing was initiated within 48 h of sample collection. In total, 619 specimens were enrolled in this study and tested with the Lyra assay on three real-time PCR platforms and with CCNA and enhanced toxigenic culture.

Direct cell culture cytotoxicity neutralization assay. An aliquot of each stool specimen was processed within 48 h of collection for direct *C. difficile* CCNA. A 1:5 dilution was prepared by adding 0.5 ml of the stool specimen to 2.0 ml of CCNA diluent. The dilution was mixed well and then centrifuged for 5 min at 2,000 × *g*. The supernatant was removed and filtered using a 0.45- μ m filter. One hundred microliters of the sample was inoculated into two wells: a control well (containing the sample and 100 μ l of *C. difficile* antitoxin) and a specimen test well (containing the sample and 100 μ l of phosphate-buffered saline). These dilutions were incubated at room temperature for 30 min. Fifty microliters of each dilution was inoculated into two separate wells of a tissue culture plate containing human foreskin fibroblasts and 150 μ l of tissue culture medium (Diagnostic Hybrids, Athens, OH). The final dilution of the specimen in each well was 1:40. Cell cultures were incubated at 35°C and were examined at 24 and 48 h. Cell rounding was used to indicate a cytotoxic effect. Specimens were considered positive for the presence of *C. difficile* cytotoxin B if at least 50% of the cells showed cytotoxic effects and if the cytotoxicity was neutralized in the control well by the antitoxin.

Enhanced *C. difficile* toxigenic cell culture. An aliquot of each stool specimen was processed within 48 h of collection for enhanced toxigenic culture. Approximately 0.1 g (1 to 2 drops) of each stool specimen was added to a prerduced chopped-meat glucose (CMG) broth (Hardy Diagnostics, Santa Maria, CA) and incubated at 35°C for 48 to 72 h. The broths were subcultured to modified cycloserine-cefoxitin-fructose agar plates with horse blood (CCFA-HB) (Remel, Lenexa, KS). CCFA-HB plates were streaked for isolation and incubated anaerobically at 35°C in a GasPak EZ anaerobe pouch system for 48 to 72 h. Following incubation, all plates were examined for colonies morphologically resembling *C. difficile* (gray-white colonies with raised centers and irregular filamentous or

opaque edges on CCFA-HB plates). All colonies resembling a *Clostridium* species were evaluated by Gram stain, vancomycin susceptibility, aerotolerance, and the presence of proline aminopeptidase. *C. difficile* was identified as strict anaerobic, Gram-positive bacilli (appearing singly or in chains), susceptible to vancomycin, positive for the presence of proline aminopeptidase, and having a characteristic "horse manure" odor. Isolates of *C. difficile* were inoculated into prerduced CMG and incubated at 35°C for 4 to 5 days. The presence of *C. difficile* toxin B in the broth culture was determined using the same CCNA test described previously with the exception of specimen preparation. For CCNA from CMG cultures, 0.5 ml of the CMG culture broth was centrifuged for 5 min at 2,000 × *g*, and then 0.2 ml of the supernatant was combined with 1.8 ml of the CCNA diluent (1:10 dilution). The diluted specimen was filtered and used in the CCNA assay as described previously.

Quidel Lyra Direct *C. difficile* assay. The Lyra assay is a real-time PCR assay performed on one of three open-platform, real-time thermocyclers (SmartCycler II [SC II], Cepheid, Sunnyvale, CA; ABI 7500 Fast DX [ABI 7500], Applied Biosystems, Carlsbad, CA; and ABI QuantStudio DX [QS DX], Applied Biosystems, Carlsbad, CA). The test is performed by placing a swab into a liquid or soft stool specimen, evenly coating it with specimen, adding it to process buffer 1, and swirling the swab. Thirty microliters of process buffer 1 containing specimen is added to process buffer 2, and the sample is then ready for real-time PCR. Each vial of lyophilized master mix is rehydrated in 135 μ l of rehydration solution (sufficient for 8 reactions), and 15 μ l of master mix is combined with 5 μ l of sample (in process buffer 2). Each specimen was processed once and used for testing with the Lyra assay on all three of the real-time PCR platforms. The results from each instrument were analyzed separately, and any invalid results were retested.

Controls. Positive and negative controls were included with each set of processed specimens. The controls were processed in the same manner as the patient specimens. The positive control contained a purified, inactivated strain of *C. difficile* (NAP1), and the negative control consisted of a *C. difficile*-free dilution matrix. The positive and negative controls had to perform as expected for the sample run to be valid. If one of the controls was invalid, the entire run was repeated.

In addition to the external controls, each sample contained an internal control, which was used to determine whether the samples were processed correctly and/or to detect the presence of PCR inhibitors in the specimen. The internal control is a component of process buffer 2 and does not have to be added to each sample individually.

Result interpretation. The Lyra assay provides a qualitative result for the presence or absence of the *tcdA* and *tcdB* genes. The test result is positive when amplification of the *tcdA* or *tcdB* gene(s) occurs regardless of whether or not amplification of the internal control occurs. The test result is negative when no amplification of the *tcdA* and *tcdB* genes occurs and amplification of the internal control is present. Test results are invalid when there is no amplification of either the *tcdA* and *tcdB* genes or the internal control. Invalid results were repeated once by retesting a new 5- μ l aliquot of sample in process buffer 2.

Data analysis and statistical methods. Results of the Lyra assay were compared to the CCNA results and to enhanced toxigenic culture using the CCNA or enhanced toxigenic culture results as the gold standard. Any results that were discordant between the Lyra assay and CCNA or enhanced toxigenic culture were considered false-positive or -negative results for the Lyra assay. The Lyra results were analyzed individually for each of the three different thermocycling platforms. The clinical sensitivity and specificity along with the 95% confidence intervals were calculated using the efficient-score method (14). The sensitivity and specificity of the Lyra assay were compared between platforms using McNemar's test (15, 16).

RESULTS

During the course of this study, 619 specimens were evaluated with the Lyra assay. Five (0.81%), nine (1.45%), and one (0.16%) of the samples were invalid on the SC II, 7500 Fast, and QS DX

TABLE 1 Overall performance characteristics of the Lyra *C. difficile* assay compared to cell culture cytotoxicity neutralization assay^a

Test site	Platform	No. of results				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		True Pos.	True Neg.	False Pos.	False Neg.				
1	Cepheid SmartCycler	11	102	12	0	100.0 (67.9–100.0)	89.5 (82.0–94.2)	47.8 (27.4–68.9)	100.0 (95.5–100.0)
	ABI 7500 Fast DX	11	102	12	0	100.0 (67.9–100.0)	89.5 (82.0–94.2)	47.8 (27.4–68.9)	100.0 (95.5–100.0)
	ABI Quant Studio DX	11	105	9	0	100.0 (67.9–100.0)	92.1 (85.1–96.1)	55.0 (32.0–76.2)	100.0 (95.6–100.0)
2	Cepheid SmartCycler	19	89	9	3	86.4 (64.0–96.4)	90.1 (82.8–95.5)	67.9 (47.6–83.4)	96.7 (90.1–99.2)
	ABI 7500 Fast DX	21	90	8	1	95.5 (75.1–99.8)	91.8 (84.1–96.2)	72.4 (52.5–86.6)	98.9 (93.2–99.9)
	ABI Quant Studio DX	20	93	5	2	90.9 (69.4–98.4)	94.9 (87.9–98.1)	80.0 (58.7–92.4)	97.9 (91.9–99.6)
3	Cepheid SmartCycler	42	295	14	5	89.4 (76.1–96.0)	95.5 (92.3–97.4)	75.0 (61.4–90.3)	98.3 (95.9–99.4)
	ABI 7500 Fast DX	44	294	10	3	93.6 (81.4–98.3)	96.7 (93.8–98.3)	81.5 (68.1–90.3)	99.0 (96.8–99.7)
	ABI Quant Studio DX	44	297	12	3	93.6 (81.4–98.3)	96.1 (93.1–97.9)	78.6 (65.2–88.0)	99.0 (96.9–99.7)
Total	Cepheid SmartCycler	72	486	35	8	90.0 (80.7–95.3)	93.3 (90.7–95.2)	67.3 (57.5–75.9)	98.4 (96.7–99.2)
	ABI 7500 Fast DX	76	491	30	4	95.0 (87.0–98.4)	94.2 (91.8–96.0)	71.7 (62.0–79.8)	99.2 (97.8–99.7)
	ABI Quant Studio DX	75	495	26	5	93.8 (85.4–97.7)	95.0 (92.7–96.7)	74.3 (64.4–82.2)	99.0 (97.5–99.6)

^a Pos., positive; Neg., negative; CI, confidence interval.

instruments, respectively. After repeating all tests, only one remained invalid. In comparison, results for three (0.48%) samples were indeterminate using CCNA due to the presence of cytotoxicity in the control well, and zero (0.00%) of the samples were indeterminate using enhanced toxigenic culture. Only the 601 results that were valid on the first attempt with all test platforms and assays were used to evaluate sensitivity and specificity.

Tables 1 and 2 summarize the test results for the Lyra assay compared to CCNA. The sensitivity with the SC II instrument was the least consistent across test sites, ranging from 86.4 to 100.0%, followed by the QS DX (90.9 to 100.0%) and the ABI 7500 (93.6 to 100.0%). Specificity was the least consistent across test sites on the ABI 7500 instrument (89.5 to 96.7%), followed by the SC II (89.5 to 95.5%) and QS DX systems (92.1 to 96.1%). The overall sensitivity (95% confidence interval) of the Lyra assay compared to that of CCNA on the SC II, ABI 7500, and QS DX instruments was 90.0% (80.7 to 95.3%), 95.0% (87.0 to 98.4%), and 93.8% (85.4 to 97.7%), respectively. The overall specificities (95% confidence interval) of the Lyra assay compared to those of CCNA on the SC II, ABI 7500, and QS DX instruments was 93.3% (90.7 to 95.2%), 94.2% (91.8 to 96.0%), and 95.0% (92.7 to 96.7%), respectively. Compared to CCNA, the ABI 7500 instrument was the most sensitive and the QS DX was the most specific, though there were no statistical differences in performance on any of the platforms (Table 2).

Tables 3 and 4 summarize the test results for the Lyra assay compared to enhanced toxigenic culture. The sensitivity with the

SCII instrument was the least consistent across test sites, ranging from 77.4 to 92.3%, followed by that with the QS DX (83.3 to 92.3%) and ABI 7500 (87.1 to 96.2%) instruments. The specificity with the ABI 7500 platform was the least consistent across sites (95.7 to 100.0%), followed by that with the SC II (95.7 to 97.3%) and QS DX (98.6 to 100.0%) instruments. The overall sensitivities (95% confidence interval) of the Lyra assay compared to enhanced toxigenic culture on the SC II, ABI 7500, and QS DX platforms were 82.1% (73.5 to 88.5%), 89.3% (81.7 to 94.1%), and 85.7% (77.5 to 91.4%), respectively. The overall specificities (95% confidence interval) of the Lyra assay compared to enhanced toxigenic culture on the SC II, ABI 7500, and QS DX platforms were 96.9% (94.9 to 98.2%), 98.8% (97.2 to 99.5%), and 99.0% (97.4 to 99.6%), respectively. Like the comparison to CCNA, the Lyra assay was the most sensitive on the ABI 7500 instrument and the most specific on the QS DX platforms compared to enhanced toxigenic culture. Unlike the comparisons of the Lyra assay to CCNA, there were significant differences in sensitivity and specificity between platforms. Results with the ABI 7500 instrument were significantly more sensitive than those with SC II ($P = 0.008$), and both the ABI 7500 and QS DX results were significantly more specific than those of the SC II assay ($P = 0.022$ and $P = 0.021$, respectively).

DISCUSSION

The Lyra assay is an FDA-approved real-time PCR assay for the detection of the *C. difficile* *tcdA* and *tcdB* genes. In this study, we compared the performance of the Lyra assay to that of two well-

TABLE 2 Comparison of Lyra *C. difficile* assay on different thermocycling platforms to cell culture cytotoxicity neutralization assay^a

Platform	Comparison		
	ABI 7500 Fast DX	ABI QuantStudio DX	Cepheid SmartCycler II
ABI 7500 Fast DX		94.2% vs 95.0%; $P = 0.424$	94.2% vs 93.3%; $P = 0.332$
ABI QuantStudio DX	93.8% vs 95.0%; $P = 1.000$		95.0% vs 93.3%; $P = 0.064$
Cepheid SmartCycler II	90.0% vs 95.0%; $P = 0.125$	90.0% vs 93.8%; $P = 0.250$	

^a Percentage from the platform in the row is listed first; that for the platform in the column is listed second. Boldface values indicate sensitivity; those in regular font indicate specificity.

TABLE 3 Overall performance characteristics of Lyra *C. difficile* assay compared to enhanced toxigenic culture^a

Test site	Platform	No. of results				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		True Pos.	True Neg.	False Pos.	False Neg.				
1	Cepheid SmartCycler	20	98	3	4	83.3 (61.8–94.5)	97.0 (90.9–99.2)	87.0 (65.3–96.6)	96.1 (89.7–98.7)
	ABI 7500 Fast DX	21	99	2	3	87.5 (66.5–96.7)	98.0 (92.3–99.7)	91.3 (70.5–98.5)	97.1 (91.0–99.2)
	ABI Quant Studio DX	20	101	0	4	83.3 (61.8–94.5)	100.0 (95.4–100.0)	100.0 (80.0–100.0)	96.2 (90.0–98.8)
2	Cepheid SmartCycler	24	90	4	2	92.3 (73.4–98.7)	95.7 (88.8–98.6)	85.7 (66.4–95.3)	97.8 (91.6–99.6)
	ABI 7500 Fast DX	25	90	4	1	96.2 (78.4–99.8)	95.7 (88.8–98.6)	86.2 (67.4–95.5)	98.9 (93.2–99.9)
	ABI Quant Studio DX	24	93	1	2	92.3 (73.4–98.7)	98.9 (93.4–99.9)	96.0 (77.7–99.8)	97.9 (91.9–99.6)
3	Cepheid SmartCycler	48	286	8	14	77.4 (64.7–86.7)	97.3 (94.5–98.7)	85.7 (73.2–93.2)	95.3 (92.1–97.3)
	ABI 7500 Fast DX	54	294	0	8	87.1 (75.6–93.9)	100.0 (98.4–100.0)	100.0 (91.7–100.0)	97.4 (94.6–98.8)
	ABI Quant Studio DX	52	290	4	10	83.9 (71.9–91.6)	98.6 (96.3–99.6)	92.9 (81.9–97.7)	96.7 (93.8–98.3)
Total	Cepheid SmartCycler	92	474	15	20	82.1 (73.5–88.5)	96.9 (94.9–98.2)	86.0 (77.6–91.7)	96.0 (93.7–97.1)
	ABI 7500 Fast DX	100	483	6	12	89.3 (81.7–94.1)	98.8 (97.2–99.5)	94.3 (87.6–97.7)	97.6 (95.7–98.7)
	ABI Quant Studio DX	96	484	5	16	85.7 (77.5–91.4)	99.0 (97.4–99.6)	95.0 (88.3–98.2)	96.8 (94.7–98.1)

^a Pos., positive; Neg., negative; CI, confidence interval.

established culture methods. Additionally, we compared the performance of the assay across all three real-time platforms which are FDA cleared for use with this assay, in parallel, using the same specimens.

Compared to CCNA, the real-time PCR platforms did not significantly vary in performance, with sensitivities and specificities ranging from 90.0 to 95.5% and 93.3 to 95.0%. However, compared to enhanced toxigenic culture, the ABI 7500 platform was significantly more sensitive than the SC II (89.3 versus 82.1%), and both the ABI 7500 and QS DX were significantly more specific than the SC II (98.8% and 99.0% versus 96.9%). There are several possible explanations for these discrepancies. The most logical reason is that the SC II performs extension at a lower temperature (66°C) than the ABI 7500 (68°C) or the QS DX (68°C), increasing the chance for nonspecific amplification. Another difference between the platforms is the filters. All of the instruments have similar optical filters, but the optimal wavelengths for each filter are not exactly the same, and even slight differences in the optics could account for the differences in the results. Finally, the QS DX and ABI 7500 utilize a 96-well plate format where the cycling and optics are consistent for all wells of the plate. The SC II platform works more like a series of individual thermocyclers. If even one SC II module at one test site was slightly out of calibration, it would be difficult to notice and could account for a few additional false results. It would take only a few additional false results to make the platform appear less sensitive or specific. Despite the fact that there are some significant differences between platforms, the

results with all platforms compare favorably to those obtained with other molecular assays for the detection of *C. difficile*, as evidenced by the product inserts and many additional clinical studies (11, 17–24).

In addition to the fact that there are some performance differences between thermocycling platforms, the Lyra assay also has a few other minor drawbacks. These include the fact that it does not identify specific *C. difficile* markers that are often associated with epidemic strains of *C. difficile* (e.g., binary toxin or Δ *tcdC* deletion mutations). However, this is only a minor concern, since none of the current infection control or treatment guidelines recommend changes in practice or treatment based solely on the strain *C. difficile* (7). Another minor drawback of this test is that it lends itself more to a “batch testing” format rather than an “on-demand testing” format. The batches can be relatively small based on the reagent packaging, but the need for inclusion of a positive and negative control with each sample makes testing most efficient and cost-effective when samples are processed simultaneously in larger batches.

Like other recently FDA-approved *C. difficile* molecular assays, the Lyra assay requires only 2 to 3 h to complete and compares favorably to enhanced toxigenic culture (sensitivity and specificity of approximately 85.7% and 98.3%, respectively, depending on the thermocycling platform used for testing). These results are relatively consistent across three test sites on all three test platforms despite the fact that each site may have different patient populations and different *C. difficile* ribotypes circulating. Finally,

TABLE 4 Comparison of Lyra *C. difficile* assay on different thermocycling platforms to enhanced toxigenic culture^a

Platform	Comparison		
	ABI 7500 Fast DX	ABI QuantStudio DX	Cepheid SmartCycler II
ABI 7500 Fast DX		98.8% vs 99.0%; <i>P</i> = 1.000	98.8% vs 96.9%; <i>P</i> = 0.022*
ABI QuantStudio DX	85.7% vs 89.3%; <i>P</i> = 0.125		99.0% vs 96.9%; <i>P</i> = 0.021*
Cepheid SmartCycler II	82.1% vs 89.3%; <i>P</i> = 0.008*	82.1% vs 85.7%; <i>P</i> = 0.219	

^a Percentage from the platform in the row is listed first; that for the platform in the column is listed second. Boldface values indicate sensitivity; those in regular font indicate specificity.

*, significant difference in performance of Lyra *C. difficile* assay between test platforms.

the only equipment required to run this assay is a real-time thermocycler. With three options to choose from, most high-complexity laboratories will already have one of these instruments available, making the initial investment more manageable. Overall, the Lyra assay has proven to be an easy-to-use, versatile molecular test for the detection of toxigenic *C. difficile* infection.

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