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EQUIVALENT PERFORMANCE OF THE COBAS® CDIFF TEST FOR USE ON THE COBAS® LIAT® SYSTEM AND THE COBAS® 4800 SYSTEM

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Clostridium difficile infection is a significant health burden, and innovative solutions are needed to shorten time to diagnosis and improve infection control. We evaluated the performance of the cobas® Cdiff test for use on the cobas® Liat® System (cobas® Liat® Cdiff), a single-sample, on-demand, and automated molecular solution with a 20-min turnaround time. The limit of detection was 45–90 colony-forming units (CFUs)/swab for toxigenic strains that covered the most prevalent toxinotypes, including the hypervirulent epidemic 027/BI/NAP1 strain. Using 442 prospectively collected clinical stool specimens, we compared the performance of the cobas® Liat® Cdiff to direct culture and to the cobas® Cdiff test on the cobas® 4800 System (cobas® 4800 Cdiff) – a medium-throughput molecular platform. The sensitivity and specificity of the cobas® Liat® Cdiff compared to direct culture were 93.1% and 95.1%, respectively, and this performance did not statistically differ from the cobas® 4800 Cdiff ($P < 0.05$). Direct correlation of the cobas® Liat® and cobas® 4800 Cdiff tests yielded overall percent agreement of 98.6%. The test performance, automation, and turnaround time of the cobas® Liat® Cdiff enable its use for on-demand and out-of-hours testing as a complement to existing batch testing solutions like the cobas® 4800 Cdiff.

Keywords: *Clostridium difficile,* PCR, POC, CDI, near-patient, molecular, NAAT

Introduction

Clostridium difficile infection (CDI) is a leading cause of healthcare-associated infection worldwide [1, 2], and the incidence and severity of cases continue to rise [3, 4]. Across Europe, the estimated mean incidence rate of CDI in the hospital is 4.1 per 10,000 patient-days and 23 infections per 10,000 hospital days [4], with an incremental cost of CDI ranging from £4577 to £8843 [5]. In the United States (US) alone, *C. difficile* causes a half-million new infections each year that are associated with an estimated 29,000 deaths, 2.4 million additional hospital days and $1.5–7 billion in excess healthcare costs [1, 6–8]. The increase in CDI has been attributed to the emergence of hypervirulent epidemic strains such 027/BI/NAP1 [9, 10] as well the increased sensitivity and utilization of newer diagnostics like nucleic acid amplification tests, or NAATs [1, 3]. In addition, the number of community-acquired CDI cases and the proportion of healthcare-associated CDI cases being diagnosed outside of the hospital setting are increasing [2, 8], signaling a need for new and different management approaches. Accurate and timely diagnosis is essential to prompt earlier treatment and enhance infection control to prevent the spread of the organism.

NAATs have become the predominantly used method for CDI diagnosis in the US due to their high sensitivity and specificity [11, 12], and their adoption is increasing throughout Europe [13]. NAAT methods, such as real-time polymerase chain reaction (PCR) targeting of *C. difficile* toxin [2], have improved the detection of *C. difficile* and reduced the need for repeat testing and empiric treatment [14]. The stand-alone use of cheaper, rapid, but less sensi-

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tive toxin enzyme immunoassays (EIA) is no longer recommended, and time-intensive reference standards such as cytotoxigenic culture are no longer standard practice [1, 2, 15, 16]. A government-sponsored comparative effectiveness review in the US concluded that the strength of evidence for diagnosing CDI using NAAT as a stand-alone test is high and exceeds toxin EIAs, glutamate dehydrogenase (GDH) testing, and multi-step testing algorithms that are gaining popularity in Europe [15].

Most diagnostic testing for CDI occurs in the centralized laboratory, but delays due to inefficient laboratory-related logistics remain a significant challenge [17–19]. Many commercially available NAATs are designed for use on laboratory platforms with moderate-high complexity and that perform batched processing of specimens [20, 21]. While the use of automated and higher throughput solutions may be preferred in light of current care models and reimbursement structures [22], operational limitations such as personnel availability or specimen processing costs can lead to longer response times [22, 23]. The mean time from test order to test result for CDI was 1.8 days in an inpatient cohort study, with a mean time from receipt in the laboratory to completion of test results being 0.4 days [18]; laboratory-related delays were attributed to specimen rejection and holding samples (often till the next day) for batch processing. Other reports suggest even longer turnaround time in the laboratory [24]. As a complement to batched and centralized testing, high-performing diagnostic solutions for CDI that can enable on-demand or off-hours testing are needed.

Recently, the cobas® Cdiff test for use on the cobas® Liat® System (cobas® Liat® Cdiff), a fast (20-min turnaround time), single-sample, easy-to-use, and compact qualitative real-time PCR test, has been introduced for clinical use [25, 26]. In this study, we evaluated the analytical and clinical performance of the cobas® Liat® Cdiff. We compared this clinical performance to a previously introduced cobas® Cdiff test that allows for medium-throughput batched sample processing on the cobas® 4800 System (cobas® 4800 Cdiff) [21, 27]. We hypothesized that the clinical performance of both cobas® Cdiff tests would be equivalent.

Materials and methods

The cobas® Liat® Cdiff is an automated, qualitative real-time PCR for the detection of the toxin B (*tcd*B) gene of *C. difficile* in unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI, providing results in approximately 20 min. Sample preparation, PCR amplification, and real-time detection of target DNA sequences are fully automated. The internal control uses *Bacillus thuringiensis israelensis,* a Gram-positive bacterium, as a full process control. In brief, the cobas® Liat® System, in conjunction with the assay tube, performs reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, PCR amplification, real-time detection, and result interpretation. When an assay tube containing a patient sample is inserted into the analyzer, multiple sample processing actuators compress the assay tube to selectively release the reagents, moving the sample from one segment to the next, and controlling reaction conditions. An embedded microprocessor controls and coordinates these actions to perform all required assay processes within the closed and self-contained assay tube, minimizing cross-contamination between samples.

The testing process has been refined to a series of simple steps: preparing a patient sample for transfer into the assay tube and capping the tube, scanning the tube's barcode, and inserting the tube into the analyzer. The system automatically executes all the required assay steps as described above and reports test results. By simplifying nucleic acid testing, the system enables non-specialized personnel to conduct sophisticated molecular diagnostic testing in a wide range of settings.

Limit of detection (analytical sensitivity) and precision

The analytical sensitivity for the cobas® Liat® Cdiff was determined using five-member test panels prepared with two different strains of *C. difficile* (ATCC 43255; R12087). Each of the test panels had *C. difficile* densities that ranged from above to below (225, 90, 45, 9, and 2.25 colony-forming units [CFUs]/swab) the expected limit of detection (LOD). Replicates of each test panel member were then tested with two different lots of assay tubes to determine the lowest *C. difficile* density where all members with that and higher densities showed a hit rate of at least 95%.

To determine the within-laboratory precision of the cobas® Liat® Cdiff, precision panels consisting of one negative and three positive densities of *C. difficile* were prepared using one cultured strain (ATCC 43255) and three different reagent lots. The negative panel member consisted of negative stool background, and the three positive panel member densities were weak positive (~0.3 × LOD; 6 CFUs/ml), low positive (~1 × LOD; 20 CFUs/ml), and moderate positive (~3 × LOD; 60 CFUs/ml). For each reagent lot, two different operators tested all four panel members in duplicate on four non-consecutive testing days; 192 replicates were tested in the study (48 replicates for each panel member) across six different analyzers. One negative control was tested on each day, and one positive control was tested each week of the study. Expected positivity rates were 0% for negative, 20%–80% for weak positive, ≥95% for low positive, and ≥99% for moderate positive panel members. Cycle threshold (Ct) values were analyzed to determine the component of variations attributed to between-lot, between-instrument, between-day, and random-error effects.

Inclusivity (toxinotype and ribotype coverage)

To verify consistent detection of all relevant toxinotypes and ribotypes of *C. difficile,* we tested 40 *C. difficile*

strains in negative stool background in triplicate using the cobas® Liat® Cdiff. Tested strains, comprised of 32 toxinotypes and 21 ribotypes, included the hypervirulent epidemic 027/BI/NAP1 strain [28] as well as the most prevalent worldwide toxinotypes of *C. difficile* – III-PCR ribotype 027, IV-PCR ribotype 023, V-PCR ribotype 078/126, and VIII-PCR ribotype 017 [29]. Twenty-seven of the 40 *C. difficile* tested strain samples were extracted genomic DNA from the respective strains (obtained from Dr. Maja Rupnik, Institute of Public Health Maribor/ National Laboratory of Health, Environment and Food, University of Maribor, Maribor, Slovenia). The remaining 13 *C. difficile* samples were generated from bacterial cultures.

Cross reactivity

To assess non-specific cross reactivity or interference of the cobas® Liat® Cdiff due to non-toxigenic strains of *C. difficile,* human genomic DNA, and other microorganisms that could be present in clinical stool specimens, we prepared test panels that contained each of these microorganisms. Each panel was tested with the cobas® Liat® Cdiff with or without a *C. difficile* organism at 3× LOD. A total of 145 organisms were tested, including Gram-positive bacterial species (i.e., *Staphylococcus aureus, Lactobacillus acidophilus, Enterococcus faecalis*) and Gram-negative bacterial species *(Escherichia coli, Enterobacter aerogenes, Bacteroides fragilis), Clostridium* spp. including 2 non-toxigenic *C. difficile* strains, relevant viruses (echo-, noro-, rota-, adeno-, and cytomegalo-virus), and HCT-15 human cells. The microorganism densities correspond to approximately 1*E* + 06 units (CFU, infection-forming unit [IFU], cells) per 1 ml of stool specimen for bacteria and human epithelial cells (as a source for human genomic DNA), and 1*E* + 05 $TCID_{50}$ (50% tissue culture infective dose) and/or plaque-forming unit (PFU) per 1 ml of stool specimen for viruses. Since the contents of a single polyester swab (containing approximately 227 mg of stool specimen) are transferred by a swab into 4.5 ml of cobas® PCR Media when preparing a specimen for a test, the amounts in stool specimen described above correspond to densities in PCR media suspension of 5*E* + 04 CFU, IFU, or cells/ml for bacteria and human epithelial cells, and 5*E* + 03 $TCID_{50}$ and/or PFU/ml for viruses.

Exogenous and endogenous interfering substances

To determine if potentially interfering exogenous substances cause false-positive or false-negative results, we tested their influence in both the absence and presence of relevant *C. difficile* strains at approximately 3× LOD. Thirty-eight potentially interfering exogenous substances were tested, including relevant over-the-counter products and antibiotic drugs such as Aleve™, Dulcolax™, glycerin suppositories, hydrocortisone, Imodium™, metronidazole, Monistat™, Pepto-Bismol™, hemorrhoidal cream and ointment, vancomycin, and local contraceptives. For evaluations, a nine-member test panel was prepared for each potential interferent and testing was performed using a single replicate of each test panel member with the cobas® Liat® Cdiff.

Similarly, potentially interfering endogenous substances including blood, mucin, and fecal fat were assessed both in the absence and presence of *C. difficile* strains at concentrations that correspond to approximately 3× LOD. Blood was tested at 100%, 50%, and 25% (*v*/*v*), mucin at 50% and 25% (*v*/*v*), and fecal fat concentrations tested ranged from 0.22% to 39%.

Clinical performance evaluations

We established the clinical performance of the cobas® Liat® Cdiff against the cobas® 4800 Cdiff and direct tissue culture cytotoxicity testing. A total of 442 anonymized stool specimens were prospectively collected and tested from two clinical sites in the US (175 from NorthShore University HealthSystem in Evanston, Illinois; 267 from TriCore Reference Laboratory in Albuquerque, New Mexico). Specimens were stored at 2 to 8 °C prior to testing.

Inclusion criteria for subjects providing specimens were as follows: 1) age >24 months, 2) meeting institutional eligibility criteria for *C. difficile* testing, 3) unformed stool sample with at least 3 ml in volume, and 4) obtaining written informed consent by subject, or a legal parent/ guardian consent if a minor (subject to requirements by local governing institutional review board [IRB]/ethics committee).

Exclusion criteria for subjects providing specimens were as follows: 1) formed stool specimen, 2) receipt of antibiotic therapy in the 14 days prior to sample collection with known activity against *C. difficile* (oral and parenteral metronidazole, oral vancomycin, fidaxomicin), 3) previous enrollment in the current study, 4) contraindication to collection of stool samples, according to the institution's policies and procedures, and 5) personally identifiable to investigator or sponsor for sites not requiring informed consent.

For each sample, one of two stool aliquots was placed into cobas® PCR media and tested on both the cobas® Liat® and cobas® 4800 Systems at Roche Molecular Diagnostics. A second stool aliquot was sent to NorthShore University HealthSystem for tissue culture cytotoxicity testing (*N* = 442). Each stool specimen was inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar (CCFA-HT). Identification of suspected colonies as *C. difficile* by Gram staining, aero-intolerance, and by the Pro-Disk Test was followed by inoculation into anaerobic chopped meat broth. Supernatants obtained from anaerobic chopped meat broth were then processed for the detection of *C. difficile* toxin B using tissue culture cytotoxicity test (C. DIFFICILE TOX-B test, Techlab, Blacksburg, VA).

Statistical analysis

For analytical performance analysis, the LOD was determined by hit rate analysis (average of the bacterial concentration levels with a ≥95% hit rate). For the precision study, the positive result rate and the 95% score binomial confidence intervals were calculated for each panel member concentration, along with an analysis of variance components and contribution to the total variance on Ct values from positive results on the cobas® Liat® Cdiff. For the clinical performance analysis of both the cobas® Liat® and cobas® 4800 Cdiff tests against the direct culture test comparison method, we calculated sensitivity, specificity, negative predictive value (NPV), and overall percent agreement (OPA). The 95% score binomial confidence intervals were calculated for each performance measurement.

To demonstrate the equivalence between the cobas® Liat® Cdiff and the cobas® 4800 Cdiff tests, we correlated the paired results of each tests and calculated the positive percent agreement (PPA), negative percent agreement (NPA), and OPA. We then measured the equivalency in sensitivity and specificity (compared to direct culture) of both molecular tests by using the two one-sided test (TOST) procedure relative to an equivalence margin of ±5% (the largest *P* value from each TOST procedure was reported). StatXact-9 software by Cytel Software Corporation was used for analyses.

Ethics

The study procedures were carried out in accordance with the Declaration of Helsinki. The institutional review board of the aforementioned institutions approved of specimen collection when applicable. All subjects who provided clinical specimens were informed about the study and all provided informed consent.

Results

Limit of detection (analytical sensitivity) and precision

The LOD was determined to be 90 CFUs/swab (20 CFUs/ml) for *C. difficile* strain 43255; hit rates for samples with 45 CFUs/ml ranged between 66.7% and 80%. The LOD for *C. difficile* strain R12087 was 45 CFUs/swab (10 CFUs/ml); hit rates for samples with 9 CFUs/ml ranged between 57.1% and 66.7%.

A total of 192 replicates were tested in the precision study, 48 replicates for each of the four panel member densities. Consistent with *a priori* expectations, positivity rates were 0% (0/48; 95% confidence interval [CI]: 0%–7.4%) for the negative panel member, 68.8% (33/48; 95% CI: 54.7%–80.1%) for the weak positive panel member, and 100% (48/48; 95% CI: 92.6%–100%) for both the low positive and moderate positive panel members. Mean Ct levels were 31.8 and 30.3 for the low positive and moderate positive concentrations, respectively. Both the low positive and moderate positive densities reported random error as the highest contributor to total variability (67% and 58%, respectively).

Inclusivity (toxinotype and ribotype coverage)

The cobas® Liat® Cdiff detected all toxigenic *C. difficile* strains tested, including 32 toxinotypes, 21 ribotypes, and one NAP1/BI/ribotype 027 hyper-virulent epidemic strain (see *Table 1*). Only three non-toxigenic toxinotype XI (XIa, XIb, and XIc) strains were not detected, all of which do not possess *tcd*B. These results demonstrate comprehensive inclusivity for toxigenic *C. difficile*.

Cross reactivity

Positive results were not generated (denoting there was no cross reactivity) for all 145 bacterial species tested, indicating that the cobas® Liat® Cdiff test does not cross react with these organisms. The initial test when *C. difficile* target was present at approximately 3× LOD for the interference from a pool of three bacterial species – *Acinetobacter lwoffii* 15309, *Alcaligenes faecalis* 35655, and *Campylobacter jejuni* 43479 – was negative in stool samples with *C. difficile*, but positive results were obtained upon retesting. In all other test panels with *C. difficile* target and non-*C. difficile* organisms, positive results were obtained as expected. These results indicate that the performance of the cobas® Liat® Cdiff was not affected by the presence of other microorganisms or human cells.

Exogenous and endogenous interfering substances

The performance of the cobas® Liat® Cdiff was not affected by 30 of 38 tested exogenous substances that included relevant over-the-counter products or antimicrobial drugs. Eight exogenous products or drugs (Dulcolax™, Pepto-Bismol™, Tums™, Vagisil™, Equate™ Natural Vegetable, Witch Hazel) caused interference at the initial test concentration of 100% swab capacity, but testing at reduced concentrations of these potential interferents did not cause interference. Furthermore, the performance in clinical stool specimens was not affected by the presence of either 100% whole blood, 50% mucin, or fecal fat ranging from 0.22 to 39%.

Clinical performance evaluations

Paired testing of the cobas® Liat® Cdiff and cobas® 4800 Cdiff was performed on 442 prospectively collected clinical stool specimens. *Table 2* provides results of the correlation between the cobas® Liat® Cdiff and cobas® 4800 Cdiff. Eighty-three specimens tested positive on both tests,

Table 1. Detection of *C. difficile* toxinotypes and ribotypes by the cobas® Liat® Cdiff

Sample ID	*C. difficile* strain	Toxino-type	Ribo-type	Hit rate (%)
1	RMSCC 11251 (ATCC no. BAA-1382; 630)	0	012	100.0
2	EX 623	I	102	100.0
3	AC 008	II	103	100.0
4	RMSCC 12827 [2004118; CDC-204118 (NAP-1)]	III	027	100.0
5	SE 844	IIIa	080	100.0
6	CH6230	IIIc	N/A	100.0
7	RMSCC 11298 (P43)	IV	N/A	100.0
8	55767	IV	023	100.0
9	RMSCC 11300 (2748-06)	V	078	100.0
10	SE 881	V	045	100.0
11	RMSCC 11302 (SE 1203)	VI	033	100.0
12	57267	VII	063	100.0
13	RMSCC 12472 (ATCC no. 43598; 1470)	VIII	017	100.0
14	RMSCC 11299 (51680)	IX	019	100.0
15	RMSCC 11304 (CCUG 8864/STCC20309)	X	036	100.0
16	RMSCC 11305 (ES 1103)**	XIa	033	0.0
17	RMSCC 11306 (6035/06)**	XIa	N/A	0.0
18	RMSCC 12414 (F14)**	XIb	N/A	0.0
19	RMSCC 11308 (F15)	XII	N/A	100.0
20	IS 25	XII	056	100.0
21	R 9367	XIII	070	100.0
22	R 10870	XIV (New XIVa)	111	100.0
23	R 9385	XV (New XIVb)	122	100.0
24	SUC36	XVI	078	100.0
25	RMSCC 11309 (No. 1313)	XVII	232	100.0
26	K095	XVIII	014	100.0
27	TR13	XIX	N/A	100.0
28	TR14	XX	N/A	100.0
29	CH6223	XXI	N/A	100.0
30	CD07-468	XXII	N/A	100.0
31	8785	XXIII (New IXc)	N/A	100.0
32	597B	XXIV	131	100.0
33	7325	XXV	027	100.0
34	7459	XXVI	N/A	100.0
35	KK2443/2006	XXVII	N/A	100.0
36	CD08-070	XXVIII	126	100.0
37	CD07-140	XXIX	056	100.0
38	ES 130	XXX	N/A	100.0
39	WA 151	XXXI	N/A	100.0
40	173070	XXXII	N/A	100.0

** *Clostridium difficile* toxinotype XI strains do not produce toxin B (*Tcd*B)

yielding a positive percent agreement of 95.4% (95% CI: 88.8%, 98.2%). A total of 353 specimens tested negative on both tests, yielding a negative percent agreement of 99.4% (95% CI: 98.0%, 99.8%). The overall percent agreement between both tests was 98.6%.

Tables 3A and *3B* provide the performance of the cobas® Liat® and cobas® 4800 Cdiff tests compared to direct toxigenic culture, respectively. For the cobas® Liat® Cdiff test, the sensitivity was 93.1% (95% CI: 84.8, 97.0) and the specificity was 95.1% (95% CI: 92.4, 96.9). Compar-

Table 2. Performance of cobas® Liat® Cdiff compared to cobas® 4800 Cdiff

		cobas® 4800 Cdiff		
		Positive	Negative	Total
cobas® Liat® Cdiff	Positive	83	2	85
	Negative	4	353	357
	Total	87	355	442
		95% confidence interval		
Percent agreement	%	Lower bound		Upper bound
Positive (PPA)	95.4	88.8		98.2
Negative (NPA)	99.4	98.0		99.8
Overall (OPA)	98.6	97.1		99.4

PPA: positive percent agreement; NPA: negative percent agreement; OPA: overall percent agreement

Table 3. Performance of cobas® Liat® and cobas® 4800 Cdiff tests compared to direct toxigenic culture

A) Performance of cobas® Liat® Cdiff compared to direct toxigenic culture

		Direct toxigenic culture			Performance statistic	% (95% CI)
		Positive	Negative	Total		
cobas® Liat® Cdiff	Positive	67	18	85	Sensitivity	93.1 (84.8–97.0)
	Negative	5	352	357	Specificity	95.1 (92.4–96.9)
	Total	72	370	442	OPA	94.8 (92.3–96.5)
					NPV	98.6 (96.8–99.4)

B) Performance of cobas® 4800 Cdiff compared to direct toxigenic culture

		Direct toxigenic culture			Performance statistic	% (95% CI)
		Positive	Negative	Total		
cobas® 4800 Cdiff	Positive	67	20	87	Sensitivity	93.1 (84.8–97.0)
	Negative	5	350	355	Specificity	94.6 (91.8–96.5)
	Total	72	370	442	OPA	94.3 (91.8–96.1)
					NPV	98.6 (96.7–99.4)

NPV: negative predictive value; OPA: overall percent agreement; CI: confidence interval

ing the cobas® Liat® and cobas® 4800 Cdiff tests, performance (relative to direct toxigenic culture) was equivalent within a margin of ±5% for sensitivity (TOST $P = 0.047$) and specificity (TOST $P < 0.0001$).

Discussion

We demonstrated that the clinical performance of the cobas® Liat® Cdiff – with a sensitivity of 93.1% and specificity of 95.1% compared to a direct toxigenic culture used as the reference standard – was statistically equivalent to the medium-throughput cobas® 4800 Cdiff in a sample of 442 prospectively collected clinical specimens. Overall test agreement between the cobas® Liat® and cobas® 4800 Cdiff tests was 98.6%. Analytical studies provided additional performance evidence of the cobas® Liat® Cdiff. The limit of detection was 45–90 CFUs/swab for toxigenic strains that covered the most prevalent toxinotypes, including the hypervirulent epidemic 027/BI/NAP1 strain. We did not observe significant interference nor cross reactivity, and positivity rates in precision testing for low- and moderate-positive *C. difficile* densities were both 100%.

The cobas® Liat® Cdiff utilizes real-time PCR technology to detect stool toxin B (*tcd*B) gene, which is the primary virulence factor present in disease-related toxinotypes and is the most common target of existing NAATs [16, 29]. The cobas® Liat® Cdiff detects the worldwide most prevalent toxinotypes of *C. difficile* (types III, IV, V, and VIII), including the hypervirulent epidemic 027/BI/NAP1 strain [29, 30]. Toxinotype XI strains, which have a very low prevalence in humans, were not detected but their significance is debated because only binary toxin, but not toxin A or B, is produced [29, 31, 32]. Findings from a whole-genome sequencing study suggest that substantial genetic heterogeneity is present among *C. difficile* isolates from infected patients [33]. While new variants will continue to emerge, the clinically relevant toxinotypes produce *tcd*B [29].

The high sensitivity and specificity of the cobas® Liat® Cdiff are consistent with published ranges for NAATs. A European meta-analysis reported a sensitivity range of 92%–97% [16] for NAATs, and a recent comparative effectiveness review in the US reported a similar range of 90%–97% [15]. The latter review showed that non-NAATs had poorer sensitivities (compared to NAATs) with wider performance ranges: 70% (range, 66%–74%) for toxin EIA, 90% (range, 78%–96%) for glutamate dehydrogenase (GDH) tests, and 73% (range, 62%–82%) for two-step testing algorithms; NAAT specificities were similar or better to toxin EIAs and GDH tests.

NAATs are an established and increasingly used method for detecting CDI, but their exact role in the diagnostic pathway and clinical interpretation are an ongoing area of debate [11, 13]. A concern is that that toxin positivity is a better predictor of more severe CDI compared to PCR positivity [14] and that no single diagnostic method can sensitively and rapidly detect free *C. difficile* toxins in stool [34]. Therefore, some are recommending a staged diagnostic approach starting with a sensitive test such as NAAT or GDH testing followed by a specific test like toxin EIA [2, 16]. However, the evidence that NAATs lead to over-diagnosis and that toxin EIAs are superior to NAATs for detecting clinically significant disease is conflicting. Senchyna and colleagues reported on the promising use of PCR Ct values to stratify and predict which NAAT-positive patients will have toxin-negative vs. toxin-positive stool [34], eliminating the potential need for a second step test.

The role of the microbiology laboratory in defining CDI is the detection of a toxigenic *C. difficile* strain in the stool sample. Sensitive detection of toxigenic *C. difficile* in a patient's stool permits the treating physician to decide if CDI is a reasonable diagnosis that requires therapy. Thus, it is arguable that the role of the microbiology laboratory is to deploy the most sensitive diagnostic for detecting toxigenic *C. difficile* in the stool sample and not to use a testing approach designed to separate patients who may be at risk for more severe disease while missing others needing treatment. Moreover, NAAT-positive patients who are asymptomatic or have toxin-negative stool may still need infection control interventions [14, 35]. NAATs will continue to play an important role in CDI diagnosis, although additional studies are still needed to further establish their performance compared GDH tests or multi-step algorithms.

The combination of diagnostic accuracy and timely diagnosis is critical for the treatment and effective containment of *C. difficile*. NAATs are amenable to both on-demand and batch testing [36], and in this study, we showed that performance of the on-demand cobas® Liat® Cdiff is equivalent to the medium-throughput cobas® 4800 Cdiff. Centralized microbiology laboratory testing models facilitate both quality control and the automated testing of a higher volume of tests. However, the time it takes from when a test is ordered until the clinician acts on a test result can be truncated with lower-throughput and on-demand testing that reduce specimen processing and transportation needs [23, 37]. The cobas® Liat® Cdiff may help to address this unmet diagnostic need by enabling on-demand and off-hours testing in the microbiology laboratory while providing higher sensitivity than other rapid tests like toxin immunoassays or glutamate dehydrogenase testing [1, 15, 16].

On-demand diagnostics for CDI will also be increasingly needed outside of the microbiology laboratory in order to isolate patients more quickly upon admission, reduce unnecessary antibiotic use or contact precautions, and improve patient outcomes by treating patients sooner [35, 38–40]. The incidence of community-associated CDI is rising and a growing proportion of hospital-acquired CDI cases are first presenting with symptoms outside of the hospital [2, 8, 33]. NAATs are increasingly being developed as rapid testing solutions at the point of care (POC), and the cobas® Liat® System can enable timely and personalized patient management and infection control when implemented at the POC [25, 41].

Limited clinical research has been published to date on the implementation of molecular POC tests outside of the microbiology laboratory [42]. Future studies should explore the clinical impact of on-demand NAATs like the cobas® Liat® Cdiff on reducing time-to-diagnosis, improving CDI management, and containing the spread of infection both inside and outside the hospital setting. Furthermore, more comprehensive health economic evaluations are needed to compare not only the use of POC versus laboratory diagnostics, but also single-modality versus multi-step testing algorithms.

Important limitations of the clinical performance analysis in this study should be considered. First, the margin used for the statistical assessment of equivalency in performance between the cobas® Liat® and cobas® 4800 Cdiff tests was limited by the sample size of this analysis. To provide an additional measure of comparison, we also calculated the overall percent agreement of results in a direct correlation of the paired results from both molecular tests. Second, prospective samples were collected from two clinical sites in the US that may not be generalizable to broader patient populations. However, both clinical sites included multiple hospitals that provided patient samples for testing and thus represented broad areas in both the upper mid-central and southern United States.

Conclusion

In conclusion, the cobas® Cdiff test for use on the cobas® Liat® System is a sensitive NAAT with excellent strain coverage that performs equivalently to the medium-throughput cobas® Cdiff test for use on the cobas® 4800 System. The easy to use, single-sample, on-demand, and automated solution with a 20-min turnaround time makes the cobas® Liat® Cdiff a valuable complement to higher throughput batch processing of specimens in the microbiology laboratory. The cobas® Liat® Cdiff can aid in reducing the time-to-diagnosis in both outpatient and hospi-

tal settings, thereby improving diagnosis and management of patients with CDI.

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Authors' contributions

All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

S.K.G. performed the study concept and design, analysis and interpretation of data, and statistical analysis. K.L. performed the study concept and design, analysis and interpretation of data, and study supervision. J.D. performed the analysis and interpretation of data, and statistical analysis. L.R.P. performed the analysis and interpretation of data and study supervision. O.L. performed the study concept and design, as well as analysis and interpretation of data.

Conflict of interest

S.G., K.L., J.D., and O.L. are employees of Roche Molecular Diagnostics. L.R.P. has received research funding from Becton Dickinson, Cepheid, Nanosphere, 3M, GeneWEAVE and Roche. He has also received compensation for consultation from Pfizer, Cepheid, and Roche.

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