DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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December 7, 2015

ROCHE MOLECULAR SYSTEMS, INC.
DAVID W. GATES, PH.D.
SENIOR DIRECTOR, REGULATORY AFFAIRS
4300 HACIENDA DRIVE
PLEASANTON CA 94588-2722

Re: K142422
Trade/Device Name: cobas Cdiff Test
Regulation Number: 21 CFR 866.3130
Regulation Name: *Clostridium difficile* Toxin Gene Amplification Assay
Regulatory Class: II
Product Code: OZN, OOI
Dated: April 16, 2015
Received: April 17, 2015

Dear Dr. Gates:

This letter corrects our substantially equivalent letter of May 20, 2015.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809**]**), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Ribhi Shawar -S

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K142422

Device Name
cobas® Cdiff Test

Indications for Use *(Describe)*
The cobas® Cdiff Test on the cobas® 4800 system is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection of the toxin B (tcdB) gene of toxigenic Clostridium difficile (C. difficile) in unformed (liquid or soft) stool specimens obtained from patients suspected of having C. difficile infection (CDI). The cobas® Cdiff Test is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	David Gates Phone: (925) 730-8237 FAX: (925) 225-0207
Date Prepared	April 16, 2015
Proprietary Name	**cobas**® Cdiff Test
Common Name	*Clostridium difficile Test*
Classification Name	21 CFR 866.3130 - *Clostridium difficile* toxin gene amplification assay 21 CFR 862.2570 - Real Time Nucleic Acid Amplification System
Product Codes	OZN, OOI
Predicate Devices	BD MAX™ Cdiff Assay, BD MAX™ Instrument, K130470
Establishment Registration	Branchburg: 2243471 Pleasanton: 3004141078 Indianapolis: 1823260

1. DEVICE DESCRIPTION

The Roche Molecular Systems (RMS) **cobas**® Cdiff Test utilizes real-time polymerase chain reaction (PCR) for the detection of *Clostridium difficile* (*C. difficile*) DNA from unformed (liquid or soft) stool specimens to aid in the diagnosis of *Clostridium difficile* infections in humans.

The **cobas**® Cdiff Test contains two major processes: (1) automated sample preparation to extract nucleic acids from the unformed stool specimens; (2) PCR amplification of target DNA sequences using *C. difficile* specific primers, and real-time detection of cleaved fluorescent-labeled *C. difficile* specific oligonucleotide detection probes. An Internal Control (IC), containing unrelated randomized DNA sequence, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

The **cobas**® Cdiff Test utilizes six reagent kits**:**

1. **cobas**® 4800 Cdiff Amplification/Detection Kit
2. **cobas**® 4800 Cdiff Controls and Cofactor Kit
3. **cobas**® 4800 System Wash Buffer Kit
4. **cobas**® 4800 System Lysis Kit 1
5. **cobas**® 4800 System Internal Control Kit 1
6. **cobas**® 4800 System Sample Preparation Kit

Required but not provided:

cobas® PCR Media and Swab Sample Kit

Sealing mat or deep well plate cover

Caps, neutral color (for recapping post-run specimens)

1.1. Target Selection

The **cobas**® Cdiff Test utilizes real-time PCR technology to detect the conserved regions of toxin B (*tcdB*) gene. Fluorogenic target specific probes are used for the detection of the amplified *C.*

difficile DNA as well as IC. Primer and probe oligonucleotide sequences were designed to select *C. difficile* conserved sequences without cross reacting with other organisms commonly found in stool specimens.

1.2. Test Principle

1.1.1. Sample Preparation

Sample preparation for the **cobas**® Cdiff Test is automated with the use of the **cobas x** 480 instrument. Unformed stool specimens are transferred into **cobas**® PCR Media by using a swab. Organisms are lysed with chaotropic agent, proteinase K, and SDS reagents. Released nucleic acids, along with added Internal Control DNA, are bound by magnetic glass particles. They are washed and then eluted into a small volume of buffer. The instrument then takes an aliquot of the eluted material and sets up the PCR reaction with an activated Master Mix.

Note: The cobas® Cdiff Test has been validated for use with the cobas® PCR Media and Swab Sample Kit. Do not use other devices or media types.

1.1.2. PCR Amplification and TaqMan® Detection

The PCR cycling steps and detection of target signal occurs in the **cobas z** 480 Analyzer. The Master Mix reagent contains primer pairs and probes for two targets: the *C. difficile* toxin B gene and Internal Control. If the target nucleic acid sequences are present, amplification with the corresponding primers will occur by a thermostable DNA polymerase, generating PCR products (amplicons). These products are detected by specific TaqMan probes containing a fluorescent dye and a quencher. Normally, the quencher suppresses the fluorescence of the dye. However, if the PCR product is present, the probe hybridizes to the product and gets cleaved by the 5' to 3' nuclease activity of the polymerase. This reaction allows the fluorescence to be emitted from the dye, and the signal is recorded in real time during each PCR cycle by the **cobas z** 480 analyzer. The signal is interpreted by the **cobas**® 4800 System Software and reported as final results.

1.3. cobas® 4800 System Description

The **cobas**® 4800 System uses the **cobas x** 480 Instrument for sample preparation, and the **cobas z** 480 Analyzer for amplification and detection. Both the **cobas x** 480 Instrument and the **cobas z** 480 Analyzer are controlled by a computer workstation running the **cobas**® 4800 System Software.

The system hardware is unchanged from that originally approved for IVD use in PMA P100020 (**cobas**® HPV Test, April 19, 2011). The software version has been updated to software release 2.1 in order to support the expanded test menu. The updated software was cleared for other currently available tests on the **cobas**® 4800 System per Special 510(K) 140887.

2. INTENDED FOR USE

The **cobas**® Cdiff Test on the **cobas**® 4800 System is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection of the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The **cobas**® Cdiff Test is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.

3. TECHNOLOGICAL CHARACTERISTICS

The primary technological characteristics and intended use of the RMS **cobas**® Cdiff Test are substantially equivalent to other legally marketed nucleic acid amplification tests intended for the qualitative detection of toxigenic *Clostridium difficile*.

As indicated in Table 1, the RMS **cobas**® Cdiff Test is substantially equivalent to technological characteristics of the predicate device. Both assays have the same intended use, and utilize nucleic acid amplification and detection technology for the detection of *C. difficile* DNA in patient specimens.

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Table 1: Similarities and Differences between the cobas® Cdiff Test and the Predicate Device

	Submitted Device: RMS cobas® Cdiff Test	**Predicate Device: BD MAX™ Cdiff Assay, K130470**
Intended Use	The **cobas®** Cdiff Test on the **cobas®** 4800 system is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection of the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The **cobas®** Cdiff Test is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.	The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having *C. difficile* infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI.
Conditions for use	For prescription use	Same
Sample Types	Liquid and soft stool specimens	Same
Subject Status	Symptomatic	Same
Sample Collection Devices	**cobas®** PCR Media and Swab Sample Kit	BD MAX™ Cdiff Sample Buffer
Analyte Targets	Toxin B (tcdB) gene	Same
Sample Preparation Procedure	Automated by **cobas® x**480	Automated by BD MAX™ System
Amplification Technology	Real-time PCR	Same
Detection Chemistry	Paired reporter and quencher fluorescence labeled probes (TaqMan Technology) using fluorescence resonance energy transfer (FRET)	Same
Controls used	Sample processing control (IC) Positive and negative control	Sample processing control Positive and negative control (optional)
Result Analysis	Based on PCR cycle threshold analysis	Same

In summary, the intended use, technology, and characteristics of the **cobas®** Cdiff Test as compared to the predicate device do not raise any new types of safety or effectiveness questions and are substantially equivalent.

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® Cdiff Test was determined by analyzing 7 toxigenic *C. difficile* strains ATCC 43255 (VPI 10463), ATCC BAA-1382 (630), CDC 204118, R12087 (CD196), 2748-06, ATCC 43598 (1470), and F15. CDC 204118 and R12087 (CD196) are BI/ NAP1/027 hyper-virulent epidemic strains. Quantified cultures were diluted into pooled negative stool specimen matrix to determine the LOD. All levels were analyzed using **cobas**® Cdiff Test with 3 unique lots of *C. difficile* specific reagents. The LOD of the test was determined as the lowest concentration exhibiting at least 95% positive rate for which all higher concentrations were greater than or equal to 95% positive rate.

The highest LOD among 3 reagent lots are shown in Table 2. The claimed LOD among the seven strains tested was 225 CFU/swab based on 95% positive rate.

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Table 2: cobas® Cdiff Test Limit of Detection

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Strain ID	Toxinotype	REA* Type	PFG† Type	Ribotype	Phenotype	LOD (CFU/swab)	
						By Positive Rate	By Probit Analysis (95% CI)
ATCC 43255 (VPI 10463)	0	N/A	N/A	087	A+B+CDT-	113	90 (66 – 311)
ATCC BAA-1382 (630)	0	R 23	N/A	012	A+B+CDT-	81	83 (62 – 145)
CDC 204118	III	BI 8	NAP1	027	A+B+CDT+	54	42 (30 – 129)
R12087 (CD196)	III	BI	NAP1	027	A+B+CDT+	54	54 (39 -126)
2748-06	V	N/A	N/A	078	N/A	54	45 (33-113)
ATCC 43598 (1470)	VIII	N/A	N/A	017	A-B+	225	130 (96 - 228)
F15	XII	N/A	N/A	N/A	N/A	54	59 (43 – 117)

* Restriction endonuclease analysis; † Pulse Field Gel

4.2. Inclusivity

The sensitivity of the **cobas**® Cdiff Test was determined for 28 additional toxigenic strains. The inclusivity panel consisted of at least three concentrations per strain in pooled negative stool specimen matrix. Forty replicates were tested for each concentration level. The LOD was

calculated as the lowest concentration level with ≥ 95 % positive rate for which all higher concentration levels show ≥ 95 % positive rate. One lot of reagents was used for this study.

The inclusivity study results are listed in Table 3.

Table 3: Toxigenic *C. difficile* Inclusivity Results

No.	Strain	Toxinotype	Ribotype	Toxin Production	LOD	Positive Rate
1	EX 623	I	102	A+ B+ CDT−	77.9	95.0%
2	AC 008	II	103	A+ B+ CDT−	77.9	95.0%
3	SE 844	IIIa	80	A+ B+ CDT+	234	100.0%
4	55767	IV	23	A+ B+ CDT+	77.9	100.0%
5	SE 881	V	45	A+ B+ CDT+	234	100.0%
6	51377	VI	N/A	A+ B+ CDT+	234	100.0%
7	57267	VII	63	A+ B+ CDT+	77.9	97.5%
8	51680	IX	19	A+ B+ CDT+	77.9	100.0%
9	8864	X	36	A− B+ CDT+	77.9	97.5%
10	R 9367	XIII	70	A+ B+CDT−	77.9	97.5%
11	R 10870	XIV	111	A+ B+ CDT+	234	100.0%
12	R 9385	XV	122	A+ B+ CDT+	234	100.0%
13	SUC36	XVI	78	A− B+ CDT+	234	100.0%
14	J9965	XVII	N/A	A− B+ CDT+	460	97.5%
15	K095	XVIII	14	A+ B+ CDT−	234	95.0%
16	TR13	XIX	N/A	A+ B+ CDT−	234	97.5%
17	TR14	XX	N/A	A+ B+CDT−	77.9	100.0%
18	CH6223	XXI	N/A	A+ B+CDT−	234	100.0%
19	CD07-468	XXII	N/A	A+ B+ CDT+	234	100.0%
20	8785	XXIII	N/A	A+ B+ CDT+	234	95.0%
21	597B	XXIV	131	A+ B+ CDT+	234	97.5%
22	7325	XXV	27	A+ B+ CDT+	234	100.0%
23	7459	XXVI	N/A	A+ B+ CDT−	234	95.0%
24	KK2443-2006	XXVII	N/A	A+ B+ CDT−	234	100.0%
25	CD08-070	XXVIII	126	A+ B+ CDT+	234	97.5%
26	CD07-140	XXIX	56	A+ B+ CDT−	234	97.5%
27	ES 130	XXX	N/A	A- B+ CDT+	234	100.0%
28	WA 151	XXXI	N/A	A- B+ CDT+	460	100.0%

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4.3. Precision

In-house precision study was conducted with *C. difficile* concentrations below Limit of Detection (LOD), near LOD and above LOD of the **cobas**® Cdiff Test including pooled negative stool specimen matrix. The study used three unique lots of **cobas**® Cdiff Test reagents and three instruments for a total of 36 runs over 12 days (3 runs per day). A description of the precision panels, the study results, and variance components are shown in Table 4. An analysis of the variance of the Ct values from valid tests was performed on positive panel members at LOD and above LOD concentrations (Table 5) and suggested that most variability of target Ct values is attributed to within run (random) and lot to lot factors (60.0% and 25.3%, respectively) for concentration level at or around LOD. For concentration level above LOD, most of the Ct value variability is attributed to within run (random) and instrument to instrument factors (72.5% and 24.7%, respectively).

Overall CV (%) at LOD and above LOD were 1.5 and 1.1%, respectively (Table 6).

Table 4: In-House Precision Study Positive Rate Analysis

Panel Member	Expected Positivity rate	No. of Negative	No. of Positive	Total	Positive Rate	95% LCL	95% UCL
Level 0	0%	72	0	72	0.0%	0.0%	5.0%
Level 1	20-80%	51	21	72	29.2%	19.0%	41.1%
Level 2	≥ 95%	0	72	72	100.0%	95.0%	100.0%
Level 3	≥ 99%	0	72	72	100.0%	95.0%	100.0%

Table 5: Variance Components Analysis for Precision Panel at or around and above LOD (Limit of Detection)

Panel Member	N	Mean	Variance Components by Factor Percent Contribution to Total					Total
			Lot	Instrument	Kit Size	Day	Random	
At or around LOD (Level 2)	72	38.5	0.0789	0.0189	0.0001	0.0270	0.1875	0.3123
			25.3%	6.0%	0.0%	8.6%	60.0%	100.0%
Above LOD (Level 3)	72	37.5	0.0047	0.0404	0.0000	0.0000	0.1188	0.1638
			2.8%	24.7%	0.0%	0.0%	72.5%	100.0%

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Table 6: Standard Deviations and Coefficients of Variation (%) Analysis for Precision Panel at or around and above LOD (Limit of Detection)

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Panel Member	N	Mean	SD Components by FactorPercent CV					Total
			Lot	Instrument	Kit Size	Day	Random	
At or around LOD (Level 2)	72	38.5	0.2808	0.1374	0.0098	0.1643	0.4330	0.5589
			0.7%	0.4%	0.0%	0.4%	1.1%	1.5%
Above LOD (Level 3)	72	37.5	0.0682	0.2009	0.0000	0.0000	0.3446	0.4047
			0.2%	0.5%	0.0%	0.0%	0.9%	1.1%

4.4. Analytical Specificity

The **cobas**® Cdiff Test was examined for analytical specificity by testing non-toxigenic *C. difficile*, other *Clostridium* genus species, human DNA, and other organisms commonly found in digestive tract in the absence and the presence of toxigenic *C. difficile* strain at ~3xLOD concentration.

In addition, analytical specificity against *C. botulinum*, which is a highly regulated national select agent, was examined by computer based *in-silico* analysis.

The **cobas**® Cdiff Test gave expected negative results in the presence of 131 organisms and human DNA. Computer based *in-silico* analysis indicated that any cross reactivity against *C. botulinum* is highly unlikely.

Table 7: Organisms for Analytical Specificity

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No.	Organism	Testing Concentration	Unit
1	*Abiotrophia defective*	1E6	CFU/mL
2	*Acinetobacter baumannii*	1E6	CFU/mL
3	*Acinetobacter lwoffii*	1E6	CFU/mL
4	*Aeromonas hydrophila*	1E6	CFU/mL
5	*Alcaligenes faecalis subsp. Faecalis*	1E6	CFU/mL
6	*Anaerococcus tetradius*	1E6	CFU/mL
7	*Bacillus cereus* (ATCC 13472)	1E6	CFU/mL
8	*Bacillus cereus* (ATCC 11778)	1E6	CFU/mL
9	*Bacteroides caccae*	1E6	CFU/mL
10	*Bacteroides merdae*	1E6	CFU/mL
11	*Bacteroides stercoris*	1E6	CFU/mL
12	*Bifidobacterium adolescentis*	1E6	CFU/mL

No.	Organism	Testing Concentration	Unit
13	*Bifidobacterium longum*	1E6	CFU/mL
14	*Campylobacter coli*	1E6	CFU/mL
15	*Campylobacter jejuni*	1E6	CFU/mL
16	*Candida albicans*	1E6	CFU/mL
17	*Candida catenulate*	1E6	CFU/mL
18	*Cedecea davisae*	1E6	CFU/mL
19	*Chlamydia Trachomatis Serovar L2*	1E6	EB/mL
20	*Citrobacter amalonaticus*	1E6	CFU/mL
21	*Citrobacter freundii*	1E6	CFU/mL
22	*Citrobacter koseri*	1E6	CFU/mL
23	*Citrobacter sedlakii*	1E6	CFU/mL
24	*Clostridium beijerinckii*	1E6	CFU/mL
25	*Clostridium bifermentans*	1E6	CFU/mL
26	*Clostridium bolteae*	1E6	CFU/mL
27	*Clostridium botulinum**	N/A	N/A
28	*Clostridium butyricum*	1E6	CFU/mL
29	*Clostridium chauvoei*	1E6	CFU/mL
30	*Clostridium difficile* (Non-toxigenic, Serogroup B)	1E6	CFU/mL
31	*Clostridium difficile* (Non-toxigenic, Serogroup I)	1E6	CFU/mL
32	*Clostridium fallax*	1E6	CFU/mL
33	*Clostridium haemolyticum*	1E6	CFU/mL
34	*Clostridium histolyticum*	1E6	CFU/mL
35	*Clostridium innocuum*	1E6	CFU/mL
36	*Clostridium methylpentosum*	1E6	CFU/mL
37	*Clostridium nexile*	1E6	CFU/mL
38	*Clostridium novyi*	1E6	CFU/mL
39	*Clostridium orbiscindens (re-named Flavonifractor plautii)*	1E6	CFU/mL
40	*Clostridium paraputrificum*	1E6	CFU/mL
41	*Clostridium perfringens*	1E6	CFU/mL
42	*Clostridium ramosum*	1E6	CFU/mL
43	*Clostridium scindens*	1E6	CFU/mL
44	*Clostridium septicum*	1E6	CFU/mL
45	*Clostridium sordellii*	1E6	CFU/mL
46	*Clostridium sphenoides*	1E6	CFU/mL
47	*Clostridium spiroforme*	1E6	CFU/mL
48	*Clostridium sporogenes*	1E6	CFU/mL
49	*Clostridium symbiosum*	1E6	CFU/mL
50	*Clostridium tertium*	1E6	CFU/mL
51	*Clostridium tetani*	1E6	CFU/mL
52	*Collinsella aerofaciens*	1E6	CFU/mL
53	*Corynebacterium genitalium*	1E6	CFU/mL
54	*Desulfovibrio piger*	1E6	CFU/mL

No.	Organism	Testing Concentration	Unit
55	*Edwardsiella tarda*	1E6	CFU/mL
56	*Eggerthella lenta*	1E6	CFU/mL
57	*Enterobacter aerogenes*	1E6	CFU/mL
58	*Enterobacter cloacae*	1E6	CFU/mL
59	*Enterococcus casseliflavus*	1E6	CFU/mL
60	*Enterococcus cecorum*	1E6	CFU/mL
61	*Enterococcus dispar*	1E6	CFU/mL
62	*Enterococcus faecalis*	1E6	CFU/mL
63	*Enterococcus faecium*	1E6	CFU/mL
64	*Enterococcus gallinarum*	1E6	CFU/mL
65	*Enterococcus hirae*	1E6	CFU/mL
66	*Enterococcus raffinosus*	1E6	CFU/mL
67	*Escherichia coli* (ATCC 11775)	1E6	CFU/mL
68	*Escherichia coli* (ATCC 25922)	1E6	CFU/mL
69	*Escherichia fergusonii*	1E6	CFU/mL
70	*Escherichia hermannii*	1E6	CFU/mL
71	*Fusobacterium varium*	1E6	CFU/mL
72	*Gardnerella vaginalis*	1E6	CFU/mL
73	*Gemella morbillorum*	1E6	CFU/mL
74	*Hafnia alvei*	1E6	CFU/mL
75	*Helicobacter fennelliae*	1E6	CFU/mL
76	*Helicobacter pylori*	1E6	CFU/mL
77	HCT-15 Human Cells	1E6	Cells/mL
78	*Klebsiella oxytoca*	1E6	CFU/mL
79	*Klebsiella pneumoniae* subsp. *pneumoniae*	1E6	CFU/mL
80	*Lactobacillus acidophilus*	1E6	CFU/mL
81	*Lactobacillus reuteri*	1E6	CFU/mL
82	*Lactococcus lactis*	1E6	CFU/mL
83	*Leminorella grimontii*	1E6	CFU/mL
84	*Listeria grayi*	1E6	CFU/mL
85	*Listeria innocua*	1E6	CFU/mL
86	*Listeria monocytogenes*	1E6	CFU/mL
87	*Mitsuokella multacida*	1E6	CFU/mL
88	*Mobiluncus curtisii*	1E6	CFU/mL
89	*Moellerella wisconsensis*	1E6	CFU/mL
90	*Morganella morganii*	1E6	CFU/mL
91	*Neisseria gonorrhoeae*	1E6	CFU/mL
92	*Peptoniphilus asaccharolyticus*	1E6	CFU/mL
93	*Peptostreptococcus anaerobius*	1E6	CFU/mL
94	*Plesiomonas shigelloides*	1E6	CFU/mL
95	*Porphyromonas asaccharolytica*	1E6	CFU/mL
96	*Prevotella melaninogenica*	1E6	CFU/mL
97	*Proteus mirabilis*	1E6	CFU/mL
98	*Proteus penneri*	1E6	CFU/mL

No.	Organism	Testing Concentration	Unit
99	*Providencia alcalifaciens*	1E6	CFU/mL
100	*Providencia rettgeri*	1E6	CFU/mL
101	*Providencia stuartii*	1E6	CFU/mL
102	*Pseudomonas aeruginosa*	1E6	CFU/mL
103	*Pseudomonas putida*	1E6	CFU/mL
104	*Ruminococcus bromii*	1E6	CFU/mL
105	*Salmonella choleraesuis* subsp. *choleraesuis*	1E6	CFU/mL
106	*Salmonella enterica subsp. arizonae* (f.k.a. *Salmonella choleraesuis ssp. arizonae*)	1E6	CFU/mL
107	*Salmonella enterica* subsp. *enterica serovar Choleraesuis*	1E6	CFU/mL
108	*Serratia liquefaciens*	1E6	CFU/mL
109	*Serratia marcescens*	1E6	CFU/mL
110	*Shigella boydii*	1E6	CFU/mL
111	*Shigella dysenteriae*	1E6	CFU/mL
112	*Shigella sonnei*	1E6	CFU/mL
113	*Staphylococcus aureus*	1E6	CFU/mL
114	*Staphylococcus epidermidis*	1E6	CFU/mL
115	*Stenotrophomonas maltophilia*	1E6	CFU/mL
116	*Streptococcus agalactiae*	1E6	CFU/mL
117	*Streptococcus dysgalactiae*	1E6	CFU/mL
118	*Streptococcus intermedius*	1E6	CFU/mL
119	*Streptococcus uberis*	1E6	CFU/mL
120	*Trabulsiella guamensis*	1E6	CFU/mL
121	*Veillonella parvula*	1E6	CFU/mL
122	*Vibrio cholera*	1E6	CFU/mL
123	*Vibrio parahaemolyticus*	1E6	CFU/mL
124	*Yersinia bercovieri*	1E6	CFU/mL
125	*Yersinia rohdei*	1E6	CFU/mL
126	Cytomegalovirus (HHV5)	2E3	IU/mL
127	Human Adenovirus 40	2.19E3	PFU/mL
128	Human Coxsackievirus A 10	1E5	PFU/mL
129	Human Echovirus 11	1E5	IU/mL
130	Human Enterovirus 71	1E5	IU/mL
131	Human Rotavirus	9.77E3	PFU/mL
132	Norovirus GII	1E5	Viral Particles/mL

* By in silico analysis.

4.5. Interference

Twenty six commonly used OTC products and antibiotic medicines, as well as whole blood, mucin, fecal fat were tested for potential interference effects with the **cobas**® Cdiff Test. All

OTC products were tested at or above the levels what could be reasonably expected to be in a fecal specimen. *C. difficile* was spiked to ~ 3 x LOD (Limit of Detection) of the **cobas**® Cdiff Test and used as targets in the tests.

No interference was observed for OTC products and fecal fat. For whole blood and mucin, no interference was observed at 25% (w/v), but 50% of mucin exhibited interference (Table 8).

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Table 8: Results from Interference Substances Testing

No.	Substance	Interpretation
1	Whole blood	No interference observed at 50% (v/v)
2	Mucin	No interference observed at 25% (w/v)*
3	Fecal Fat	No interference observed up to 28% (w/v)
4	Tums	No interference observed at 10% (w/v)
5	Vancomycin	No interference observed at 1% (w/v)
6	Metronidazole	No interference observed at 10% (w/v)
7	Imodium AD®	No interference observed at 10% (w/v)
8	Stool Softener	No interference observed at 10% (w/v)
9	Pepto-Bismol® (Procter & Gamble)	No interference observed at 10% (v/v)
10	Nystatin Ointment USP	No interference observed at 10% (w/v)
11	Preparation H® with Bio-Dyne® Cream (Wyeth)	No interference observed at 10% (w/v)
12	GYNOL II	No interference observed at 10% (w/v)
13	Vagisil® Anti-itch cream	No interference observed at 10% (w/v)
14	Anusol® Plus	No interference observed at 10% (w/v)
15	Sunscreen	No interference observed at 1% (w/v)
16	Monistat®7	No interference observed at 10% (w/v)
17	VaselineTM	No interference observed at 10% (w/v)
18	SAB-Dimenhydrinate® Suppositories (SABEX®)	No interference observed at 10% (w/v)
19	Mineral Oil	No interference observed at 10% (v/v)
20	Equate Natural Vegetable Laxative	No interference observed at 10% (w/v)
21	Dulcolax®	No interference observed at 10% (w/v)
22	Fleet® (CB Fleet Company)	No interference observed at 10% (w/v)
23	K-Y Jelly/Gelée® (McNeil-PPC)	No interference observed at 1% (w/v)
24	Afrin Original Nasal Spray	No interference observed at 10% (v/v)
25	Witch hazel	No interference observed at Liquid from 1 wipe
26	E-Z-HD™ High Density Barium Sulfate for suspension (E-Z-EM Canada)	No interference observed at 20% (w/v)
27	Palmitic acid	No interference observed at 10% (w/v)
28	Steric acid	No interference observed at 10% (w/v)
29	Aleve	No interference observed at 10% (w/v)

*Mucin at 50% (v/v) concentration interfered with the detection of toxigenic *C. difficile* isolates.

4.6. Cross Contamination

The "worst case use scenario" cross contamination rate for the **cobas**® Cdiff Test was assessed by testing high titer C. difficile and negative samples that were processed in a checkerboard configuration on cobas® 4800 system. High titer samples were prepared by adding C. difficile culture to pooled negative stool specimen matrix to generate a Ct of the 95 percentile of the clinical specimen population.

Five runs were performed on each of three **cobas**® 4800 systems (total: 15 runs). The first run on each system contained only the negative samples to confirm the instrument was clean. The subsequent three runs on each system had alternating the positive and negative samples in checkerboard configurations to assess the cross contamination rate. The last run on each system contained only the negative samples to assess the carry-over contamination rate.

Results from this study are summarized in Table 9. In the nine checkerboard runs, 1 out of 423 negative samples exhibited a positive result, for an observed cross contamination rate of 0.24%. All results in the last 3 runs containing only the negative samples were negative, suggesting that there was no carry-over run-to-run contamination.

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Table 9: Cross Contamination and Carry-over Contamination Rate

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Run Type	No. of Runs	Total Negative Samples	Number of Positive Results in Negative Samples	Contamination Rate
Checkerboard run (Cross Contamination)	9	423	1	0.24%
Last run with negative panel (Carryover Contamination)	3	282	0	0.00%

5. CLINICAL PERFORMANCE EVALUATION

5.1. Reproducibility

The reproducibility of the **cobas**® Cdiff Test on the **cobas**® 4800 System was established in a multi-site investigation using simulated clinical samples evaluated across lot, site/instrument, operator, day and within-run.

Reproducibility test panels consisting of 4 specimens, with 3 replicates each, were prepared by seeding pooled, *C. difficile*-negative, unformed stool in **cobas**® PCR Media with varying concentrations of *C. difficile* strain ATCC 43255 (Negative, Below LOD, 1 x LOD, and 3 x LOD) and tested at 3 sites by 2 operators/day for 5 days/lot over 2 lots, for a total of 720 tests per panel member (4 specimens x 3 replicates x 3 sites x 2 operators/site x 5 days/lot x 2 lots).

The results are summarized in Table 9 and Table 10. Overall, 60 runs were performed; all were valid. Of the 720 test performed across 4 panel members (Negative, Below LOD, 1 x LOD, 3 x LOD), there were 712 (98.9%) valid results; 7 failed results were due to clot detection or pipetting errors, and 1 invalid result due to IC dropout. All valid test results were included in percent agreement analyses.

Table 10 summarizes the Ct values and the percent agreement (two-sided 95% exact CI) by site and panel member. The SD and CV (%) for Ct values across positive panel members ranged from 0.64 to 0.71 and 1.7 to 1.9%, respectively. The positive percent agreement for the *C. difficile* positive panel members "Below LOD," "1 x LOD," and "3 x LOD" were 66.1% (95% CI: 58.7% to 73.0%), 100.0% (95% CI: 98.0% to 100.0%), and 100.0% (95% CI: 97.9% to 100.0%), respectively. The negative percent agreement for negative panel members was 100.0% (95% CI: 97.9% to 100.0%).

Table 10: Summary of Reproducibility Results: Ct Values and Percent Agreement by Site and Panel Member

Panel Member	Valid Tests Results (n)	Ct			Percent Agreement by Site (n/N)			Total Agreement	
		Mean	SD	CV (%)	1	2	3	Percent (n/N)	(95% CI)b
Negative	174	N/A	N/A	N/A	100.0 (60/60)	100.0 (60/60)	100.0 (54/54)	100.0% (174/174)	(97.9%, 100.0%)
Below LOD	180	39.7	0.71	1.8	71.7 (43/60)	68.3 (41/60)	58.3 (35/60)	66.1% (119/180)	(58.7%, 73.0%)
1 x LOD	180	37.6	0.64	1.7	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)
3 x LOD	178	36.6	0.70	1.9	100.0 (60/60)	100.0 (60/60)	100.0 (58/58)	100.0% (178/178)	(97.9%, 100.0%)

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Table 11 presents the SD and CV (%) of Ct values for positive panel members overall and attributable to lot, site/instrument, operator, day, and within-run.

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Table 11: Overall Mean, Standard Deviations, and Coefficient of Variation (%) for Ct Values from Valid Results for Positive Panel Members

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			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	**N**	**Mean Ct**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Below LOD	119	39.7	0.33	0.8%	0.00	0.0%	0.12	0.3%	0.21	0.5%	0.58	1.5%	0.71	1.8%
1 x LOD	180	37.6	0.54	1.4%	0.08	0.2%	0.00	0.0%	0.06	0.1%	0.33	0.9%	0.64	1.7%
3 x LOD	178	36.6	0.60	1.7%	0.13	0.4%	0.10	0.3%	0.09	0.3%	0.29	0.8%	0.70	1.9%

Ct = cycle threshold; CV = coefficient of variation; Inst. = instrument; LOD = limit of detection; SD = standard deviation.

5.2. Clinical Performance

The clinical performance of the **cobas®** Cdiff Test was established in an IRB-approved, prospective, multi-site, investigation comparing the results with toxigenic culture using leftover, de-identified, unformed stool samples from subjects suspected of having CDI. Specimens were collected at five geographically diverse sites across the US from symptomatic eligible male and female subjects. The toxigenic culture was performed at a single reference laboratory and the **cobas®** Cdiff Test was performed at one of three designated sites. The toxigenic culture included direct and repeat direct and enrichment culture of stool followed by cytotoxicity testing. The direct culture included the transfer of sample to pre-reduced selective anaerobic media, cycloserine-cefoxitin-fructose agar with horse blood and taurocholate (CCFA-HT), followed by cytotoxicity testing on *C. difficile* recovered from stool. Briefly, suspected colonies obtained from direct cultures were identified as *C. difficile* by Gram stain, aerotolerance test, and by the Pro Disk test (Hardy Diagnostics, Santa Maria, CA) and then inoculated into anaerobic chopped meat broth and incubated for 5 to 7 days at 35°C for cytotoxicity testing. Supernatants obtained from anaerobic chopped meat broth were then processed for the detection of *C. difficile* toxin B using cell culture cytotoxicity testing (*C. DIFFICILE TOX-B TEST*, TECHLAB®). Enriched toxigenic

culture included culture using cycloserine-cefoxitin-manitol broth with taurocholate, lysozyme and cysteine (CCMB-TAL), followed by subculture on *Brucella* agar plates, and with identification and cytotoxicity testing of *C. difficile* recovered from enrichment culture as described. A specimen was considered positive for toxigenic *C. difficile* if *C. difficile* was recovered from stool either by direct or enriched toxigenic culture and if isolates recovered tested positive by cytotoxicity testing (any positive rule). If *C. difficile* was isolated from the direct culture and the isolate tested positive by cell cytotoxicity assay, the enrichment culture was not further analyzed. Specimens were classified as negative for toxigenic *C. difficile* only if they tested negative by both direct, and repeat direct and enrichment culture. The sensitivity, specificity, and PPV and NPV values were calculated by comparing **cobas®** Cdiff Test results with the combined results of direct and enrichment toxigenic culture. Discrepant analysis was performed on all samples with discordant results, and a random subset of specimens with concordant results, between the **cobas®** Cdiff Test and toxigenic culture, using a second FDA-cleared nucleic acid amplification test (NAAT). In addition, the positive percent agreement (PPA) and negative percent agreement (NPA) was determined comparing the **cobas®** Cdiff Test with the initial direct culture results.

Results

Specimens were collected from 683 subjects; 306 males (44.8%) and 377 females (55.2%) with a mean age of 56 years (range 3 to 99). Specimens from all 683 subjects had valid results for both direct toxigenic culture and the **cobas®** Cdiff Test but one sample lacked sufficient volume for repeat direct and enrichment culture and was not included in the statistical analysis. Of the 683 specimens, 113 were positive for toxigenic *C. difficile* during the initial direct toxigenic culture and 141 of 682 were positive for toxigenic *C. difficile* using the combined results from the initial direct and repeat direct and enrichment toxigenic culture, for a prevalence rate of 20.7% for the study.

Comparison with combined direct and enrichment culture

The clinical performance of the **cobas®** Cdiff Test compared with the combined results of initial direct and repeat direct and enriched toxigenic culture are shown in Table 13.

The sensitivity and specificity of the **cobas®** Cdiff Test was 92.9% (131/141; 95% CI: 87.4% to 96.1%) and 98.7% (534/541; 95% CI: 97.4% to 99.4%), respectively; and the PPV and NPV was 94.9% (95% CI: 89.9% to 97.5%) and 98.2% (95% CI: 96.6% to 99.0%), respectively. Of the 10 specimens with false-negative **cobas®** Cdiff Test results relative to combined direct culture and enrichment culture, all 10 were negative by a second NAAT method. Of the 7 specimens with false-positive **cobas®** Cdiff Test results relative to combined direct and enrichment culture, 3 were positive and 4 were negative by that second NAAT method.

Table 13: Comparison of cobas® Cdiff Test with combined direct culture and enrichment culture

		Combined Direct and Enrichment Culture[a]		
		Positive	Negative	Total
cobas® Cdiff Test	Positive	131	7[c]	138
	Negative	10[b]	534	544
	Total	141	541	682
	Sensitivity:	92.9% (131/141; 95% CI = 87.4% to 96.1%)		
	Specificity:	98.7% (534/541; 95% CI = 97.4% to 99.4%)		
	PPV:	94.9% (95% CI = 89.9% to 97.5%)		
	NPV:	98.2% (95% CI = 96.6% to 99.0%)		

[a] Includes combined results from an initial direct culture and a repeat direct and enrichment culture performed on all initial direct culture-negative samples. One specimen with an initial direct culture-negative result had insufficient specimen volume to perform repeat direct culture and enrichment culture and was excluded from the analysis. Thirty-six (36) specimens with initial direct culture-negative results had their combined direct and enrichment culture results based on repeat culture that used three culture plate media (CCFA, CCFA-HB, CCFA-VA) in combination with enrichment culture. Of these 36 specimens, 21 were culture positive.

[b] Of the 10 specimens with false-negative **cobas®** Cdiff Test results relative to combined direct and enrichment culture, all 10 were negative by a second NAAT method

[c] Of the 7 specimens with false-positive **cobas®** Cdiff Test results relative to combined direct and enrichment culture, 3 were positive and 4 were negative by that second NAAT method.

Comparison with direct culture

The performance of the **cobas®** Cdiff Test compared to initial direct culture is shown in 14. The PPA and NPA of the **cobas®** Cdiff Test compared to the initial direct culture for all 683 subjects was 97.3% (110/113) and 94.9% (541/570), respectively. Of the 3 specimens with false-negative **cobas®** Cdiff Test results relative to direct culture, all 3 were negative by a second NAAT method. Of the 29 specimens with false-positive **cobas®** Cdiff Test results relative to direct culture, 15 were positive and 13 were

negative by that second NAAT method; 1 sample was not tested because of insufficient specimen volume.

Table 14: Comparison of cobas® Cdiff Test with direct culture

		Direct Culture		
		Positive	Negative	Total
cobas® Cdiff Test	Positive	110	29[b]	139
	Negative	3[a]	541	544
	Total	113	570	683
Positive Percent Agreement:		97.3% (110/113; 95% CI = 92.5% to 99.1%)		
Negative Percent Agreement:		94.9% (541/570; 95% CI = 92.8% to 96.4%)		

[a] Of the 3 specimens with false negative **cobas**® Cdiff Test results relative to direct culture, all 3 were negative by a second NAAT method.

[b] Of the 29 specimens with false positive **cobas**® Cdiff Test results relative to direct culture, 15 were positive, 13 were negative by that second NAAT method, and 1 sample was not tested because of insufficient specimen volume.

5.3. Summary

The clinical performance evaluation as documented in the reproducibility and clinical study support the conclusion that the **cobas**® Cdiff test is as safe and effective as the predicate device.

6. CONCLUSIONS

A comparison of the intended use, technological characteristics, and the results of non-clinical and clinical performance studies support the conclusion that the **cobas**® Cdiff Test is substantially equivalent to the predicate device.