DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

September 12, 2017

Roche Molecular Systems, Inc.
Clare Santulli
Senior Manager
4300 Hacienda Drive
Pleasanton, California 94588-2722

Re: K171770
Trade/Device Name: cobas Cdiff Nucleic acid test for use on the cobas Liat System
Regulation Number: 21 CFR 866.3130
Regulation Name: *Clostridium difficile* toxin gene amplification assay
Regulatory Class: Class II
Product Code: OZN, OOI
Dated: June 13, 2017
Received: June 14, 2017

Dear Clare Santulli:

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 Part 801 and Part 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 regulation (21 CFR Part 801 and Part 809), please contact the Division of Industry and Consumer Education (DICE) 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 (DICE) 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,

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)*
K171770

Device Name
cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System

Indications for Use *(Describe)*
The cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the 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 Nucleic acid test for use on the cobas® Liat® System 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.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	Clare Santulli Phone: (925) 730-8886 FAX: (925) 225-0207 Email: clare.santulli@roche.com
Date Prepared	September 11, 2017
Proprietary Name	**cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System
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	**cobas**® Cdiff Test on the **cobas**® 4800 system
Establishment Registration	Branchburg: 2243471 Pleasanton: 3004141078 Indianapolis: 1823260

1. DEVICE DESCRIPTION

The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System (**cobas**® Cdiff) is a rapid, automated *in vitro* diagnostic test for the qualitative detection of *C. difficile* DNA in human stool specimens.

The **cobas**® Liat® System is for *in vitro* diagnostic use. The system is designed to identify and/or measure the presence of genetic material in a biological sample. The system automates all nucleic acid amplification test (NAAT) processes, including reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification, real-time detection, and result interpretation in a rapid manner.

1.1. Target Selection

The **cobas**® Cdiff test detects the *tcdB* target-specific and IC-specific oligonucleotide sequences. Toxin B (or tcdB) is a major toxin that is implicated in *C. difficile* pathogenesis and allows the differentiation between toxigenic and non-toxigenic *C. difficile* strains. Primers and probe oligonucleotide sequences were designed to detect *C. diff*icile conserved sequences without cross-reacting with other *Clostridium* genus organisms, as well as with organisms commonly found in normal gut flora.

1.2. Test Principle

The **cobas**® Cdiff test uses silica magnetic particle-based nucleic acid extraction and TaqMan probe-based real-time PCR amplification and detection. The **cobas**® Liat® Analyzer automates and integrates sample purification, nucleic acid amplification and detection of the target sequence in biological samples. Other than adding the sample to the **cobas**® Cdiff assay tube, no reagent preparation or additional steps are required. The **cobas**® Liat® System consists of an **cobas**® Liat® Analyzer with integrated software for running tests and analyzing the results, and a single-use disposable **cobas**® Cdiff assay tube that holds all of the sample purification and PCR reagents and hosts the sample preparation and PCR processes specific for the Cdiff analyte. The test uses the assay tube as both the sample and reaction vessel. The assay tube comprises flexible tubing containing all required unit dose reagents pre-packed in tube segments, separated by pressure-sensitive seals, in the order of reagent use.

During the testing process, multiple sample processing actuators of the analyzer compress the **cobas**® Cdiff assay tube to selectively release reagents from tube segments, move the sample from one segment to another, and control reaction conditions such as reaction volume, temperature, pressure, and incubation time. Precise control of all these parameters provides optimal conditions for assay reactions, allowing the test to achieve high performance similar to or better than that of currently available molecular assays. The **cobas**® Liat® Analyzer software controls and coordinates these actions to perform all required assay processes, including sample preparation, nucleic acid extraction, target enrichment, inhibitor removal, nucleic acid elution, and real-time PCR. All assay steps are performed within the closed and self-contained **cobas**® Cdiff assay tube, thereby eliminating the potential for cross-contamination between samples. The collected data are automatically analyzed and the result is displayed in the assay report on the integrated LCD touch screen of the **cobas**® Liat® Analyzer.

2. INDICATIONS FOR USE

The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is an automated, qualitative *in vitro* diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the detection of the toxin B (*tcd*B) 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 Nucleic acid test for use on the **cobas**® Liat® System 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 Nucleic acid test for use on the **cobas**® Liat® System are substantially equivalent to other legally marketed nucleic acid amplification tests intended for the qualitative detection of *Clostridium difficile*. As indicated in Table 1, the RMS **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is substantially equivalent to significant characteristics of the identified predicate device, the currently cleared **cobas**® Cdiff Test for use on the **cobas**® 4800 System, 510(k) 142422. The predicate device and the submitted device both utilize the **cobas**® PCR Media Uni Swab Sample Kit for specimen transfer into **cobas**® PCR Media.

Table 1: Similarities and Differences between the cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System and the Predicate Device

	Submitted Device: cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System	**Predicate Device: cobas® Cdiff Test for use on the cobas® 4800 System , K142422**
Intended Use	The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the 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 Nucleic acid test for use on the **cobas**® Liat® System is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.	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.
Conditions for use	For prescription use	Same
Regulation Number, Classification and Subsequent Product Codes	21 CFR 866.2660: Microorganism differentiation and identification device. OMN: *C. difficile* Nucleic Acid Amplification Test Assay. OOI: Real Time Nucleic Acid Amplification System.	Same
Sample Types	Unformed soft stool specimens	Same
Amplification Technology	Real-time PCR	Same
Detection Technology	TaqMan probes with fluorescent dyes	Same
Internal Control	A gram-positive *Bacillus thuringiensis israelensis* bacterial organism to monitor the full process on the **cobas**® Liat® Analyzer. Native sequence in the bacteria is used as the Internal Control target.	Lambda phage with encapsulated internal control sequence
Positive Control	Plasmid in buffer	Same
Negative Control	Buffer only	Same
Analyte Targets	Toxin B (*tcd*B) gene	Same
Sample Transfer Devices	**cobas**® PCR Media Uni Swab Sample Kit	Same
Sample Preparation	Magnetic bead-based nucleic acid extraction automated by **cobas**® Liat® Analyzer	Magnetic bead-based nucleic acid extraction automated by **cobas**® 4800 System
Result Analysis	Based on PCR cycle threshold analysis	Same
Subject Status	Symptomatic	Same

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 2 toxigenic *C. difficile* strains ATCC 43255(VPI 10463) and R12087 (CDI 196). Five-member test panels were prepared with the two different strains of Cdiff at concentrations that bracketed the expected Limit of Detection (LOD). Replicates of each test panel member were then tested with two different lots of **cobas**® Liat® Cdiff assay tubes to determine the Limit of Detection, or the lowest Cdiff concentration to yield a Hit Rate of at least 95%, where all higher concentrations also have a Hit Rate of ≥ 95%. Additional replicates were tested to confirm the LOD.

Negative stool background samples were also run to verify the Limit of Blank (LoB).

The LOD results among 2 reagent lots are shown in Table 2.

Table 2: LOD cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System

Strain ID	Toxinotype	REA* Type	PFG† Type	Ribotype	Phenotype	LOD (CFU/swab)
ATCC 43255 (VPI 10463)	0	N/A	N/A	87	A+B+CDT-	90
R12087 (CD196)	III	BI	NAP1	27	A+B+CDT+	45

*Restriction endonuclease analysis; †Pulse Field Gel

4.2. Inclusivity

The limit of detection of **cobas**® Cdiff test on 37 toxigenic strains representing additional toxinotypes was verified by testing three replicates per strain at three times the LOD level (~60 CFU/ml equivalent to 270 CFU/swab) of ATCC 43255.

All 37 toxigenic strains were detected with a 100% hit rate for each toxigenic strain in this study, confirming that the **cobas**® Cdiff test can detect these *C. difficile* toxinotypes. Results are shown in Table 3.

Table 3: Results of Testing Three Replicates of 40 Cdiff Strains at 60CFU/mL (~3x LOD)

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

4.3. Analytical Specificity (Cross-reactivity)

The **cobas**® Liat® Cdiff test was examined for analytical specificity by testing 146 non-toxigenic strains (toxinotype XI) of *Clostridium difficile*, human genomic DNA, and other microorganisms which could be present in clinical stool specimens at ~3xLOD concentration.

Test panels were prepared that contained each of these microorganisms, and each test panel was tested with the **cobas**® Liat® Cdiff assay tubes. The microorganism concentrations that were tested correspond to approximately 1E+06 units (CFU, IFU, cells) per mL of stool specimen for bacteria and human epithelial cells (as a source for human genomic DNA) and 1E+05 $TCID_{50}$and/or PFU per mL of stool specimen for viruses.

Three non-toxigenic Cdiff strains (toxinotype XI) tested during inclusivity study [RMSCC # 11305 (ES 1103), 11306 (6035/06) and 12414 (F14)] were not detected by the **cobas**® Cdiff test are included in this section.

The **cobas**® Liat Cdiff Test did not cross react with Cdiff non-toxigenic (toxinotype XI) strains, human epithelial cells (tested as a source of human genomic DNA) or other microorganisms which could be present in clinical stool specimens and that were tested in the study. Further, the presence of any of these potential cross reactants tested did not interfere with detection of toxigenic Cdiff strains tested at approximately 3x LOD level.

All 149 non-toxigenic strains (toxinotype XI) of *Clostridium difficile*, human genomic DNA, and other microorganisms are listed in Table 4.

Table 4: All Cross Reactivity Panel Members

Organism / Cell Number	Organism / Cell Name:	Source#
1	*Abiotrophia defectiva*	ATCC# 49176
2	*Acinetobacter baumannii*	ATCC# 19606
3	*Acinetobacter lwoffii*	ATCC# 15309
4	*Aeromonas hydrophila*	ATCC# 7966
5	*Alcaligenes faecalis*	ATCC# 35655
6	*Alcaligenes faecalis subsp. Faecalis*	ATCC# 8750
7	*Alcaligenes faecalis subsp. Faecalis*	ATCC# 15554
8	*Anaerococcus tetradius*	ATCC# 35098
9	*Bacillus cereus*	ATCC# 13472
10	*Bacillus cereus*	ATCC# 11778 (also known as HER 1414)
11	*Bacteroides caccae*	ATCC# 43185
12	*Bacteroides fragilis*	ATCC# 25285
13	Bacteroides merdae	ATCC# 43184
14	*Bacteroides stercoris*	ATCC# 43183
15	*Bifidobacterium adolescentis*	ATCC# 15703
16	*Bifidobacterium longum*	ATCC# 15707
17	*Campylobacter coli*	ATCC# 33559
18	*Campylobacter jejuni (f.k.a Campylobacter coli)*	ATCC# 43479
19	*Campylobacter jejuni Subsp. jejuni*	ATCC# 33292
20	*Candida albicans*	ATCC# 10231
21	*Candida catenulata*	ATCC# 10565
22	*Cedecea davisae*	ATCC# 33431
23	*Chlamydia Trachomatis Serovar L2*	Zeptometrix # 0801776
24	*Citrobacter amalonaticus*	ATCC# 25405
25	*Citrobacter freundii*	ATCC# 8090
26	*Citrobacter koseri*	ATCC# 27028
27	*Citrobacter sedlakii*	ATCC# 51115
28	*Clostridium beijerinckii*	ATCC# 8260
29	*Clostridium bifermentans*	ATCC# 638
30	*Clostridium bolteae*	ATCC# BAA-613
31	*Clostridium butyricum*	ATCC# 19398
32	*Clostridium chauvoei*	ATCC# 11957
33	Clostridium difficile (Non-toxigenic, Serogroup B)	ATCC# 43593
34	*Clostridium difficile (Non-toxigenic, Serogroup I)*	ATCC# 43601
35	*Clostridium fallax*	ATCC# 19400
36	Clostridium haemolyticum	ATCC# 9650
37	*Clostridium histolyticum*	ATCC# 19401
38	*Clostridium innocuum*	ATCC# 14501
39	Clostridium methylpentosum	ATCC# 43829
40	Clostridium nexile	ATCC# 27757
41	Clostridium novyi	ATCC# 19402
42	*Clostridium orbiscindens (re-named Flavonifractor plautii)*	ATCC# 49531

Organism / Cell Number	Organism / Cell Name:	Source#
43	*Clostridium paraputrificum*	ATCC# 17796
44	*Clostridium perfringens*	ATCC# 13124
45	*Clostridium ramosum*	ATCC# 25582
46	Clostridium scindens	ATCC# 35704
47	Clostridium septicum	ATCC# 12464
48	Clostridium sordellii	ATCC# 9714
49	Clostridium sphenoides	ATCC# 19403
50	*Clostridium spiroforme*	ATCC# 29899
51	*Clostridium sporogenes*	CCRI# 11128
52	*Clostridium sporogenes*	ATCC# 15579
53	Clostridium symbiosum	ATCC# 14940
54	*Clostridium tertium*	DSMZ# 662
55	*Clostridium tetani*	ATCC# 19406
56	*Collinsella aerofaciens*	ATCC# 25986
57	*Corynebacterium genitalium*	ATCC# 33030
58	Cytomegalovirus (AD-169)	ZeptoMetrix # 0810003CF
59	*Desulfovibrio piger*	ATCC# 29098
60	*Edwardsiella tarda*	ATCC# 15947
61	Eggerthella lenta	ATCC# 25559
62	*Enterobacter aerogenes*	ATCC# 13048
63	*Enterobacter cloacae*	ATCC# 13047
64	*Enterococcus casseliflavus*	ATCC# 25788
65	*Enterococcus cecorum*	ATCC# 43198
66	*Enterococcus dispar*	ATCC# 51266
67	*Enterococcus faecalis Van B*	ATCC# 51299
68	*Enterococcus faecium Van A*	ATCC# 35667
69	*Enterococcus gallinarum Van C*	ATCC# 49573
70	*Enterococcus hirae*	ATCC# 8043
71	*Enterococcus raffinosus*	ATCC# 49427
72	*Escherichia coli*	ATCC# 11775
73	*Escherichia coli*	ATCC# 25922
74	*Escherichia coli O157:H7*	ATCC# 700927
75	*Escherichia fergusonii*	ATCC# 35469
76	*Escherichia hermannii*	ATCC# 33650
77	*Fusobacterium varium*	ATCC# 8501
78	*Gardnerella vaginalis*	ATCC# 14018
79	Gemella morbillorum	ATCC# 27824
80	*Hafnia alvei*	CMCC# 147
81	*HCT-15 Human Cells*	ATCC# CCL-225
82	Helicobacter fennelliae	ATCC# 35683
83	*Helicobacter pylori*	ATCC# 43504
84	Human Adenovirus 41	ZeptoMetrix # 0810085CF
85	Human Coxsackievirus A4	ZeptoMetrix # 0810142CF
86	Human Coxsackievirus B4	ZeptoMetrix # 0810075CF
87	Human Echovirus 11	ZeptoMetrix # 0810023CF

Organism / Cell Number	Organism / Cell Name:	Source#
88	Human Enterovirus 71	ZeptoMetrix # 0810236CF
89	Human Rotavirus	ZeptoMetrix # 0810041CF
90	*Klebsiella oxytoca*	ATCC# 33496
91	*Klebsiella pneumoniae subsp. Pneumoniae*	ATCC# 13883
92	*Lactobacillus acidophilus*	ATCC# 4356
93	*Lactobacillus reuteri*	ATCC# 23272
94	*Lactococcus lactis*	ATCC# 19435
95	*Leminorella grimontii*	ATCC# 33999
96	*Listeria grayi*	ATCC# 19120
97	*Listeria innocua*	ATCC# 33090
98	*Listeria monocytogenes*	ATCC# 15313
99	*Listeria monocytogenes*	ATCC# BAA-839
100	*Mitsuokella multacida*	ATCC# 27723
101	*Mobiluncus curtisii*	ATCC# 35241
102	*Molellerella wisonsensis*	ATCC# 35017
103	*Morganella morganii*	ATCC# 25830
104	*Neiserria gonorrhoeae*	ATCC# 35201
105	Norovirus GII	ZeptoMetrix # 0810087CF
106	*Peptoniphilus asaccharolyticus*	ATCC# 14963
107	*Peptostreptococcus anaerobius*	ATCC# 27337
108	*Plesiomonas shigelloides*	ATCC# 14029
109	*Porphyromonas asaccharolytica*	ATCC# 25260
110	*Prevotella melaninogenica*	ATCC# 25845
111	*Proteus mirabilis*	ATCC# 29906
112	*Proteus mirabilis*	ATCC# 25933
113	*Proteus penneri*	ATCC# 35198
114	*Providencia alcalifaciens*	ATCC# 9886
115	*Providencia rettgeri*	ATCC# 9250
116	*Providencia stuartii*	ATCC# 29914
117	*Pseudomonas aeroginosa*	ATCC# 35554
118	*Pseudomonas aeruginosa*	ATCC# 33584
119	*Pseudomonas putida*	ATCC# 12633
120	*Ruminococcus bromii*	ATCC# 27255
121	*Salmonella enterica subsp. arizonae (f.k.a. Salmonella choleraesuis ssp. arizonae)*	ATCC# 13314
122	*Salmonella enterica subsp. enterica*	CMCC# 1975
123	*Salmonella enterica subsp. enterica serovar Choleraesuis*	ATCC# 7001
124(^)	*Salmonella enterica subsp. enterica serovar Typhi*	ATCC# 19430
125	*Salmonella enterica subsp.enterica serovar Typhimurium*	ATCC# 14028
126	*Serratia liquefaciens*	CMCC# 169
127	*Serratia marcescens*	ATCC# 8100
128	*Serratia marcescens*	ATCC# 13880
129	*Serrratia liquefaciens*	ATCC# 27592

Organism / Cell Number	Organism / Cell Name:	Source#
130	*Shigella boydii*	ATCC# 9207
131	*Shigella dysenteriae*	ATCC# 11835
132	*Shigella sonnei*	ATCC# 29930
133	*Staphylococcus aureus*	ATCC# 43300
134	*Staphylococcus epidermidis*	ATCC# 14990
135	*Stenotrophomonas maltophilia*	ATCC# 13637
136	*Streptococcus agalactiae*	ATCC# 13813
137	*Streptococcus dysgalactiae*	ATCC# 43078
138	*Streptococcus intermedius*	ATCC# 27335
139	*Streptococcus sp.; strain V8*	ATCC# 12973
140	*Streptococcus uberis*	ATCC# 19436
141	*Trabulsiella guamensis*	ATCC# 49490
142	*Veillonella parvula*	ATCC# 10790
143	*Vibrio cholerae*	ATCC# 25870
144	*Vibrio parahaemolyticus*	ATCC# 17802
145	*Yersinia bercovieri*	ATCC# 43970
146	*Yersinia rohdei*	ATCC# 43380
147	*Clostridium difficile* (Non-toxigenic- XIa) (ES 1103)*	BMTU# 13799 (RMSCC 11305)
148	*Clostridium difficile* (Non-toxigenic- Xia) (6035/06)*	BMTU#9961 (RMSCC 11306)
149	*Clostridium difficile* (Non-toxigenic-XIb) (F14)*	BMTU# 9962 (RMSCC 12414 and 11307)

* Non-toxigenic *Clostridium difficile* (#147,148,149) were tested during Inclusivity study.

4.4. Interference

Thirty eight commonly used medications, as well as fecal fat, whole blood, and mucin, were tested for potential interference effects with the **cobas**® Cdiff test. All substances were tested at levels above what could be reasonably expected to be collected by a swab in a stool specimen. The amount of interference substance is expressed as concentration in primary stool specimen. Two toxigenic C. difficile isolates were spiked to 3 x Limit of Detection (LOD) of the **cobas**® Cdiff test and used as targets in the tests. Exogenous substances at the highest tolerable concentration with no interference on **cobas**® Cdiff test are shown in Table 5. Exogenous substances concentrations higher than listed in Table 5 may generate false negative or invalid results.

For fecal fat, no interference was observed up to 39% (w/v), and for mucin, no interference was observed up to 50% (w/v). For whole blood, no interference was observed up to 100% (v/v), which is equivalent to 100% of the capacity of the transfer swab. These results are summarized in Table 5.

Table 5: Results from Interference Substances Testing

Substance	Primary Stool Specimen Concentration
Fecal Fat	0.22% - 39% (w/v)
Whole blood	100% (v/v)
Mucin	50% (w/v)
Aleve	100% (w/v)
Mylanta	100% (w/v)
Anusol	100% (w/v)
Dulcolax	23% (w/v)*
Equate Laxative	50% (w/v)*
Equate Hydrocortisone	100% (w/v)
E-Z-HD Barium Sulfate	100% (w/v)
Fleet	100% (w/v)
Glycerin Suppositories	100% (w/v)
Gravol Suppositories	100% (w/v)
Gynol II Contraceptive	10% (w/v)*
Imodium	100% (w/v)
Kaopectate	100% (w/v)
K-Y Jelly	100% (w/v)
Metronidazole	100% (w/v)

Substance	Primary Stool Specimen Concentration
Miconazole	100% (w/v)
Mineral Oil	100% (w/v)
Monistat Cream	100% (w/v)
Monistat Complete Care	100% (w/v)
Nystatin Ointment	100% (w/v)
Palmitic Acid	100% (w/v)
Pedia Lax	100% (w/v)
Pepto Bismol	25% (w/v)*
Witch Hazel	50% (w/v)*
Preparation H Hemorrhoidal Cream	100% (w/v)
Preparation H Hemorrhoidal ointment	100% (w/v)
Dramamine	12.5% (w/v)*
Steric Acid	100% (w/v)
Docusate Sodium	100% (w/v)
Tums	50% (w/v)*
Mesalamine Rectal Suspension	100% (w/v)
Vagisil Anti-itch Cream	12.5% (w/v)*
Vancomycin	100% (w/v)
Vaseline	100% (w/v)
Sun Screen	100% (w/v)
Monistat Vaginal Insert	100% (w/v)
Vaginal Contraceptive Film	1 film vortexed with 20 ml of primary stool sample
Spermicidal Condoms	1 Condom vortexed with 20 mL of primary stool sample

5. CLINICAL PERFORMANCE EVALUATION

5.1. Reproducibility

The reproducibility of the **cobas**® Cdiff test was established in a multi-site investigation (2 external sites, 1 internal site) using simulated clinical samples evaluated across reagent lot, site, operator, and testing day. Overall, 818 tests were performed in this study, out of which 798 were valid for the study and included in the final percent agreement analysis.

Panels consisted of 3 members: 1 negative specimen and 2 specimens with different concentrations of 1 strain of toxigenic C. *difficile* – the first a low positive concentration at ~ 1 x limit of detection (LOD) and the second a moderate positive at ~3 x LOD. There were

3 replicates per panel member; testing of 1 replicate sample was 1 test. A run was defined as testing of 3 replicates of a panel member. For each of 3 lots, panels were run on 5 different nonconsecutive days by 2 different operators at each of the 3 sites. Table 6 shows the percent agreement results by panel member concentration, negative percent agreement for negative panel members and positive percent agreement for positive panel members. For the ~1 x LOD panel members (low positive), the overall percent agreement was 98.5% with a lower bound of the two-sided 95% Score CI of 96.2%. Overall percent agreement was 99.3% for the ~3 x LOD panel member (moderate positive) and 100% for the negative panel member.

Table 6: Percent Agreement by Panel Member Compared to Acceptance Criteria

		Percent Agreement		
Panel Member	**Number of Valid Test Results**	**Estimate**	**(95% CI)***	**Met Acceptance Criteria**
Negative	262	100.0% (262/262)	(98.6%, 100.0%)	n/a
~1xLOD	266	98.5% (262/266)	(96.2%, 99.4%)	Yes
~3xLOD	270	99.3% (268/270)	(97.3%, 99.8%)	n/a

* 95% CI = two-sided 95% Score binomial confidence interval.

Results were in agreement when a positive panel member had a valid result of Positive (Cdiff Detected) for the analyte orwhen the negative panel member had a valid result of Negative (Cdiff Not Detected) for the analyte. n/a = not applicable

Table 7 presents the mean, total standard deviation (SD), and total percent coefficient of variation (CV %) of Ct values by panel member concentration.

Table 7 also presents percent agreement separately by lot, site, operator, and testing day.

Table 7: Percent Agreement by Panel Member for Lot, Site, Operator and Testing Day

Panel Member		Negative	~1x LOD	~3x LOD
Number of Valid Test Results		262	266	270
Overall Percent Agreement, % (n/N)*				
Overall	**Percent Agreement**	100.0 (262/262)	98.5 (262/266)	99.3 (268/270)
	95% Score CI	(98.6, 100.0)	(96.2, 99.4)	(97.3, 99.8)
Percent Agreement for Component Variables, % (n/N)*				
Reagent Lot	**1**	100.0 (85/85)	100.0 (87/87)	100.0 (90/90)
	2	100.0 (88/88)	100.0 (90/90)	100.0 (90/90)
	3	100.0 (89/89)	95.5 (85/89)	97.8 (88/90)
Site	**1**	100.0 (85/85)	100.0 (88/88)	100.0 (90/90)
	2	100.0 (89/89)	97.8 (87/89)	98.9 (89/90)
	3	100.0 (88/88)	97.8 (87/89)	98.9 (89/90)
Operator	**1**	100.0 (43/43)	100.0 (44/44)	100.0 (45/45)
	2	100.0 (42/42)	100.0 (44/44)	100.0 (45/45)
	3	100.0 (45/45)	97.7 (43/44)	100.0 (45/45)
	4	100.0 (44/44)	97.8 (44/45)	97.8 (44/45)
	5	100.0 (44/44)	100.0 (44/44)	97.8 (44/45)
	6	100.0 (44/44)	95.6 (43/45)	100.0 (45/45)
Testing Day	**1**	100.0 (53/53)	100.0 (54/54)	100.0 (54/54)
	2	100.0 (54/54)	96.3 (52/54)	100.0 (54/54)
	3	100.0 (52/52)	96.0 (48/50)	98.1 (53/54)
	4	100.0 (52/52)	100.0 (54/54)	98.1 (53/54)
	5	100.0 (51/51)	100.0 (54/54)	100.0 (54/54)

Note: CI = confidence interval.

* For the negative panel member: Percent agreement = (number of not detected results/total valid results) x 100.
For the positive panel members: Percent agreement = (number of detected results/total valid results) x 100.

Table 8 below presents the overall SD and percent CV (%) of Ct values for positive panel members at ~1 x LOD and ~3 x LOD concentrations, as well as the variance attributed to individual components (lot, site, operator, testing day, and within-run). Within-run variation refers to the variation within a 'study run' that consists of the 3 replicates for a given panel member processed by the same operator on the same analyzer on the same day.

Across all components, the total CV (%) was ≤ 1.9% with respect to the cycle threshold (Ct) value for all positive panel members. Within each component, the CV (%) was ≤ 1.6% across positive panel members.

Table 8: Overall Mean, Standard Deviation (SD) and Percent Coefficients of Variation (CV) for Ct Values from Valid Results for Positive Panel Members

			Standard Deviation and Percent Coefficient of Variation											
			Lot		Site		Operator		Day		Within-Run		Total	
Panel Member	N	Mean Ct	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
~1xLOD	262	31.4	0.13	0.4%	0.26	0.8%	0.00	0.0%	0.13	0.4%	0.51	1.6%	0.60	1.9%
~3xLOD	268	30.0	0.23	0.8%	0.25	0.8%	0.08	0.3%	0.00	0.0%	0.38	1.3%	0.51	1.7%

5.2. Clinical Performance

The clinical performance of the **cobas**® Cdiff test was established in a prospective, multi-site investigation designed to evaluate the sensitivity and specificity of the **cobas**® Cdiff test compared to the combined results of direct and enriched toxigenic culture using leftover, de-identified, unformed stool samples from patients suspected of having *C. difficile* infection.

Nine (9) study sites from geographically diverse locations participated in this study. Nineteen (19) operators distributed across these study sites performed testing with the **cobas**® Cdiff test. Reference culture (combined direct and enriched toxigenic culture) was performed at a reference laboratory. The remnant sample was stored frozen for discrepant analyses or possible additional testing, as determined by Roche Molecular Solution (RMS).

All evaluable discordant specimens and an equal number of randomly selected and representative concordant samples were used for discrepant testing by an FDA-cleared comparator NAAT. Testing was performed at an external lab that was preselected and qualified by RMS.

Evaluable fresh remnant specimens were prospectively collected from 1,013 patients; 483 males (47.7%) and 530 females (52.3%) with a median age of 59 years (range 5 to 98). All 1,013 specimens had valid results for both combined direct and enrichment culture and the **cobas**® Cdiff test. Of the 1,013 specimens, 179 were positive for toxigenic *C. difficile* using the combined results from direct and enrichment toxigenic culture, for a prevalence rate of 17.7% for the study.

5.3. Comparison with Combined Direct and Broth Enrichment Culture

The clinical performance of the **cobas**® Cdiff test compared with the combined results of direct and broth enrichment toxigenic culture are shown in Table 9. The sensitivity and specificity of the **cobas**® Cdiff test were 87.2% (156/179; 95% CI: 81.5% to 91.3%) and 98.1% (818/834; 95% CI: 96.9% to 98.8%), respectively; and the PPV and NPV were 90.7% (95% CI: 85.4% to 94.2%) and 97.3% (95% CI: 95.9% to 98.2%), respectively. Of the 23 specimens with false-negative **cobas**® Cdiff test results relative to combined direct culture and enrichment culture, 19 were negative, 3 were positive and 1 was invalid by the second NAAT method. Of the 16 specimens with false-positive **cobas**® Cdiff test results relative to combined direct and enrichment culture, 14 were positive and 2 were negative by the second NAAT method.

Table 9: Comparison of cobas® Liat® Cdiff Test with Combine Direct and Enrichment Culture Results

	Combined Direct and Enrichment Culture Result		
cobas® Liat Cdiff Test Result	**Positive**	**Negative**	**Total**
Detected	156	16	172
Not Detected	23	818	841
Total	179	834	1013
Sensitivity (95% CI)	87.2% (156/179; 95% CI = 81.5% to 91.3%)		
Specificity (95% CI)	98.1% (818/834; 95% CI = 96.9% to 98.8%)		
PPV (95% CI)	90.7% (156/172; 95% CI = 85.4% to 94.2%)		
NPV (95% CI)	97.3% (818/841; 95% CI = 95.9% to 98.2%)		

Note: Specimens with both combined direct and enrichment culture and valid cobas Liat Cdiff test results are considered evaluable and included in this summary table.
Note: CI = (score) confidence interval, PPV = positive predictive value, NPV = negative predictive value, OPA = overall percent agreement.

Of the 1,016 specimens tested with the **cobas**® Cdiff test, 1.4% were initially invalid and 0.2% initially had failed results. Following 1 retest per invalid or failed result, the final invalid rate was 0.1% and the final failed rate was 0%.

5.4. Comparison with Direct Culture

The performance of the **cobas**® Cdiff test compared to direct culture is shown in Table 10. The positive percent agreement (PPA) and negative percent agreement (NPA) of the **cobas**® Cdiff test compared to the direct culture for all 1,013 specimens were 94.6% (139/147) and 96.2% (833/866), respectively. Of the 8 specimens with false-negative **cobas**® Cdiff test results relative to direct culture, 7 were negative and 1 was positive by a second NAAT method. Of the 33 specimens with false-positive **cobas**® Cdiff test results relative to direct culture, only 17 were tested with the second NAAT method: 14 were positive and 3 were negative by that second NAAT method. The remaining 16/33 specimens were positive by enrichment culture and hence not tested with the second NAAT method per the discrepant analysis protocol.

Table 10: Comparison of cobas® Liat® Cdiff Test with Direct Culture Results

	Direct Culture Result		
cobas Liat Cdiff Test Result	**Positive**	**Negative**	**Total**
Detected	139	33	172
Not Detected	8	833	841
Total	147	866	1013
PPA	94.6% (139/147; 95% CI = 89.6% to 97.2%)		
NPA	96.2% (833/866; 95% CI = 94.7% to 97.3%)		

Note: Specimens with both direct culture and valid **cobas** Liat Cdiff test results are considered evaluable and included in this summary table.
Note: CI = (score) confidence interval.

6. CONCLUSIONS

A comparison of the intended use, technological characteristics, and the results of non-clinical analytical and clinical performance studies demonstrate that the **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is substantially equivalent to the predicate device.