

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K142422

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

tcdB gene of toxigenic *Clostridium difficile*

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) assay

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

cobas[®] Cdiff Test

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3130, *Clostridium difficile* toxin gene amplification assay

21 CFR 862.2570 - Instrumentation for clinical multiplex test systems

2. Classification:

II

3. Product code:

OZN, OOI

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) 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. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

cobas[®] 4800 System

I. Device Description:

The Roche Molecular Systems (RMS) cobas Cdiff Test utilizes real-time polymerase chain reaction (PCR) for the detection of toxigenic *Clostridium difficile* (*C. difficile*) DNA from unformed (liquid or soft) stool specimens to aid in the diagnosis of *C. 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 RMS cobas Cdiff Test is designed to be performed on the cobas 4800 System. The cobas 4800 utilizes the cobas x480 Instrument for automated sample preparation, and the cobas z480 Analyzer for automated amplification and detection. The cobas 4800 system software integrates the sample preparation with nucleic acid amplification and detection to generate test results

Materials and reagents provided:

- cobas 4800 Cdiff Amplification/Detection Kit
- cobas 4800 Cdiff Controls and Cofactor Kit
- cobas 4800 System Wash Buffer Kit
- cobas 4800 System Lysis Kit 1
- cobas 4800 System Internal Control Kit 1
- cobas 4800 System Sample Preparation Kit

Additional materials required but not provided:

- CORE Tips, 1000 µL, rack of 96
- 50 mL Reagent Reservoir
- 200 mL Reagent Reservoir
- cobas 4800 System Extraction (deep well) Plate
- cobas 4800 System AD (microwell) Plate 0.3 mL and Sealing Film
- Sealing foil applicator
- 24-position carrier
- Solid waste bag
- Hamilton STAR Plastic Chute
- cobas PCR Media and Swab Sample Kit
- Disposable gloves, powderless
- Vortex Mixer (single tube)
- Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500

J. Substantial Equivalence Information:

1. Predicate device name(s):

BD MAX™ Cdiff Assay

2. Predicate 510(k) number(s):

K130470

3. Comparison with predicate:

Similarities		
Item	Device cobas Cdiff Test	Predicate BD MAX <i>C.diff</i> 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 (<i>tcdB</i>) gene of toxigenic <i>Clostridium difficile</i> (<i>C. difficile</i>) in unformed (liquid or soft) stool specimens obtained from patients suspected of having <i>C. difficile</i> 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 <i>C. diff</i> Assay performed on the BD MAX System is an automated in vitro diagnostic test for the direct, qualitative detection of the <i>Clostridium difficile</i> toxin B gene (<i>tcdB</i>) in human liquid or soft stool specimens from patients suspected of having <i>C. difficile</i> infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of <i>C. difficile</i> toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX <i>C. diff</i> Assay is intended to aid in the diagnosis of CDI.
Measurand	<i>C. difficile</i> toxin B gene (<i>tcdB</i>)	Same
Specimen Type	unformed (liquid or soft) stool specimens	Same
Amplification technology	Real-time PCR	Same
Detection Technology	TaqMan [®] probes	Same
Sample extraction	Automated	Same

Differences		
Item	Device cobas Cdiff Test	Predicate BD MAX <i>C.diff</i> Assay (K130470)
Instrument platform	cobas 4800 System	BD MAX System
Assay Controls	Sample processing control (IC) Positive and negative	Sample processing control (IC) Positive and negative

Differences		
Item	Device cobas Cdiff Test	Predicate BD MAX <i>C.diff</i> Assay (K130470)
	control	control (optional)
Sample transfer device	Polyester swab	10µL inoculating loop

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

Sample preparation for the cobas Cdiff Test is automated with the use of the cobas x480 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.

The PCR cycling steps and detection of target signal occurs in the cobas z480 Analyzer. The Master Mix reagent contains primer pairs and probes for two targets: *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. 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 z480 analyzer. The signal is interpreted by the cobas 4800 System Software and reported as final results.

The cobas 4800 System uses the cobas x480 Instrument for sample preparation, and the cobas z480 Analyzer for amplification and detection. Both the cobas x480 Instrument and the cobas z480 Analyzer are controlled by a computer workstation running the cobas 4800 System Software.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision

An in-house precision study was conducted using a panel composed of *C. difficile*

cultures diluted into negative stool matrix in cobas PCR Media to concentration levels below Limit of Detection (LOD), near LOD and above LOD of the cobas Cdiff Test. A negative level composed of only the stool suspension in cobas PCR Media was also tested. The study used three unique lots of cobas Cdiff Test reagents and three instruments for a total of 36 runs over 12 days. A description of the precision panels and the study summary is shown in Table 1. Analysis of the variance components (Table 2) suggested that most variability of target Ct values was attributed to within run and lot to lot factors (60.0% and 25.3%, respectively) for concentrations at or around LOD. For concentration levels above LOD, most of the Ct value variability was attributed to within run and instrument to instrument factors (72.5% and 24.7%, respectively). Overall percent coefficients of variation (CV) for panel members at LOD and above LOD were 1.5% and 1.1%, respectively (Table 3).

Table 1. Precision study positive rate analysis

Panel Member	N Tested	N Positive	Positive Rate	95% CL	
				Lower	Upper
Negative	72	0	0.0%	0.0%	5.0%
< 1 x LOD	72	21	29.2%	19.0%	41.1%
~ 1 x LOD	72	72	100.0%	95.0%	100.0%
~ 3 x LOD	72	72	100.0%	95.0%	100.0%

LOD = Limit of Detection

Table 2. Variance components analysis for precision panel members

Level	Mean Ct	Variance of Components/Percent Contribution to Total					Total
		Lot	Instrument	Kit Size	Day	Within Run	
~ 1 x LOD	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%
~ 3 x LOD	37.5	0.0047	0.0404	0.0000	0.0000	0.1188	0.1638
		2.8%	24.7%	0.00%	0.00%	72.5%	100.0%

LOD = Limit of Detection

Table 3. Standard deviations and percent CV for precision panel members

Level	Mean	SD of Components/Percent CV					Total
		Lot	Instrument	Kit Size	Day	Within Run	
~ 1 x LOD	38.5	0.28	0.14	0.01	0.16	0.43	0.56
		0.7%	0.4%	0.0%	0.4%	1.1%	1.5%
~ 3 x LOD	37.5	0.07	0.20	0.00	0.00	0.34	0.40
		0.2%	0.5%	0.0%	0.0%	0.9%	1.1%

LOD = Limit of Detection

In-house precision study results were acceptable.

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 of four specimens, with three replicates each, were prepared at varying concentrations of *C. difficile* strain ATCC 43255 (Negative, Below LOD, 1 x LOD, and 3 x LOD) into pooled, *C. difficile*-negative, unformed stool matrix in cobas PCR Media and tested at three sites by two operators/day for five days/lot over two lots for an overall total of 720 tests (4 specimens x 3 replicates x 3 sites x 2 operators/site x 5 days/lot x 2 lots) or 180 tests/panel member or 90 tests/panel member/lot.

Overall, 60 runs were performed; all external controls and all runs were valid. Of the 720 test performed across all panel members, there were 712 (98.9%) valid results; Seven failed results were due to clot detection or pipetting errors, and one invalid result was due to an invalid internal control result. Only valid test results were included in percent agreement analyses.

Table 4 summarizes the Ct values and the overall 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 agreements 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 member was 100.0% (95% CI: 97.9% to 100.0%).

Table 4. 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)
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%)

Table 5. Summary of reproducibility results: percent agreement by variable and panel member

		Negative	Below LOD	1 x LOD	3 x LOD
Lot	1	100.0% (85/85)	65.6% (59/90)	100.0% (90/90)	100.0% (90/90)
	2	100.0% (89/89)	66.7% (60/90)	100.0% (90/90)	100.0% (88/88)
Site/ Instrument	1	100.0% (60/60)	71.7% (43/60)	100.0% (60/60)	100.0% (60/60)
	2	100.0% (60/60)	68.3% (41/60)	100.0% (60/60)	100.0% (60/60)
	3	100.0% (54/54)	58.3% (35/60)	100.0% (60/60)	100.0% (58/58)
Operator	1	100.0% (30/30)	76.7% (23/30)	100.0% (30/30)	100.0% (30/30)
	2	100.0% (30/30)	66.7% (20/30)	100.0% (30/30)	100.0% (30/30)
	3	100.0% (30/30)	66.7% (20/30)	100.0% (30/30)	100.0% (30/30)
	4	100.0% (30/30)	70.0% (21/30)	100.0% (30/30)	100.0% (30/30)
	5	100.0% (24/24)	53.3% (16/30)	100.0% (30/30)	100.0% (29/29)
	6	100.0% (30/30)	63.3% (19/30)	100.0% (30/30)	100.0% (29/29)
Day	1	100.0% (35/35)	69.4% (25/36)	100.0% (36/36)	100.0% (35/35)
	2	100.0% (35/35)	61.1% (22/36)	100.0% (36/36)	100.0% (36/36)
	3	100.0% (34/34)	58.3% (21/36)	100.0% (36/36)	100.0% (36/36)
	4	100.0% (35/35)	63.9% (23/36)	100.0% (36/36)	100.0% (35/35)
	5	100.0% (35/35)	77.8% (28/36)	100.0% (36/36)	100.0% (36/36)

* For the negative panel member, percent agreement = (number of negative results/total valid results) x 100; for the positive panel members, percent agreement = (number of positive results/total valid results) x 100. CI = confidence interval; Ct = cycle threshold; CV = coefficient of variation; LOD = limit of detection; N/A = not applicable; SD = standard deviation.

Table 6. Overall Mean, SD, and CV(%) for valid results from positive panel members

			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.

Reproducibility study results were acceptable.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Specimen stability

Unformed stool specimen stability was demonstrated by testing ten *C. difficile* positive and two negative clinical specimens on the cobas 4800 system after consecutive storage at $30 \pm 1^\circ\text{C}$ for two days, followed by $2-8^\circ\text{C}$ for five days, followed by -20°C for 60 days. The results supported the storage of unformed stool specimens at $2-30^\circ\text{C}$ for two days, or $2-8^\circ\text{C}$ for seven days, and at -20°C for 60 days.

The stability of stool specimens mixed with the cobas PCR media was demonstrated by testing the specimen panel mixed in the cobas PCR media after storage at $30 \pm 1^\circ\text{C}$ for seven days and at $2-8^\circ\text{C}$ for one and two months. The results supported the storage of stool specimen mixed with cobas PCR media at 30°C for seven days or at $2-8^\circ\text{C}$ for 60 days.

Controls

The cobas Cdiff Test uses Positive (+) and Negative (-) Controls (cobas 4800 Cdiff Controls and Cofactor Kit), and an Internal Control (cobas 4800 System Internal Control Kit 1). One set of Positive and Negative Controls are included in each run. For any run, valid results must be obtained for both the Positive and Negative Control for the cobas 4800 Software to display the reportable cobas Cdiff Test results from that run.

Positive control. The Cdiff (+) Control contains non-infectious DNA plasmids of *C. difficile*. The Cdiff (+) Control monitors nucleic acid extraction, amplification, and detection steps in a given run of the test. The Cdiff (+) Control result must be 'Valid'.

Negative control. The (-) Control result must be 'Valid'.

Internal control. The Internal Control is a recombinant bacteriophage lambda that contains randomized sequences and targets for internal control-specific primers and probe. The Internal Control is added to all specimens and the Positive and Negative Controls during sample preparation on the cobas x 480 instrument. The Internal Control monitors nucleic acid extraction, amplification, and detection steps for a given specimen. The Internal Control is also required for validation of the run controls.

d. *Detection limit:*

The analytical sensitivity (Limit of Detection or LOD) for the cobas Cdiff Test was determined by analyzing quantified cultures of seven toxigenic *C. difficile* strains diluted into pooled negative stool specimen matrix in cobas PCR Media. All levels were analyzed using cobas Cdiff Test across three unique reagent lots. At least 21

replicates per reagent lot were tested at each level. The LOD of the test was defined as defined as the target concentration which was detected as positive in $\geq 95\%$ of the replicates tested, based on results generated by the worst performing reagent lot. The seven *C. difficile* strains tested are shown in Table 7.

Table 7. cobas Cdiff Test Limit of Detection

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

Inclusivity/reactivity

The sensitivity of the cobas Cdiff Test was determined for 28 additional strains of toxigenic *C. difficile* tested at a minimum of three concentrations and 40 replicates per concentration level. The lowest levels that had at least a 95% hit rate are summarized in Table 8. All 28 strains were detected as positive in $\geq 95\%$ of replicates at concentrations ranging from 77.9 to 460 CFU/swab.

Table 8. Inclusivity/reactivity

<i>C. difficile</i> Strain	Toxinotype	Ribotype	Conc. (CFU/swab)	Positive Rate
EX 623	I	102	77.9	95.0%
AC 008	II	103	77.9	95.0%
SE 844	IIIa	080	234	100.0%
55767	IV	023	77.9	100.0%
SE 881	V	045	234	100.0%
51377	VI	N/A	234	100.0%
57267	VII	063	77.9	97.5%
51680	IX	019	77.9	100.0%
8864	X	036	77.9	97.5%
R 9367	XIII	070	77.9	97.5%
R 10870	XIV	111	234	100.0%
R 9385	XV	122	234	100.0%

<i>C. difficile</i> Strain	Toxinotype	Ribotype	Conc. (CFU/swab)	Positive Rate
SUC36	XVI	078	234	100.0%
J9965	XVII	N/A	460	97.5%
K095	XVIII	014	234	95.0%
TR13	XIX	N/A	234	97.5%
TR14	XX	N/A	77.9	100.0%
CH6223	XXI	N/A	234	100.0%
CD07-468	XXII	N/A	234	100.0%
8785	XXIII	N/A	234	95.0%
597B	XXIV	131	234	97.5%
7325	XXV	027	234	100.0%
7459	XXVI	N/A	234	95.0%
KK2443-2006	XXVII	N/A	234	100.0%
CD08-070	XXVIII	126	234	97.5%
CD07-140	XXIX	056	234	97.5%
ES 130	XXX	N/A	234	100.0%
WA 151	XXXI	N/A	460	100.0%

e. *Analytical specificity:*

Cross reactivity:

Cross reactivity was evaluated with a panel that consisted of 103 bacteria, fungi, and viruses that may be found in stool specimens and one human cell line (Table 9). In addition, 28 *Clostridium* genus organisms including non-toxigenic *C. difficile* were tested (Table 10).

Bacteria were quantified as colony forming units (CFU)/mL, human cells were quantified as cells/mL, and viruses were quantified as plaque forming units (PFU)/mL, except for the following microorganisms: *Chlamydia trachomatis* was quantified as elementary bodies (EB)/mL, and Cytomegalovirus, Human Echovirus, and Human Enterovirus were quantified as IU/mL.

All bacteria and human cells were spiked to 1×10^6 Units/mL, and all viruses were spiked to 1×10^5 Units/mL, except for Adenovirus Type 40, Cytomegalovirus (HHV5), and Human Rotavirus, which were spiked to lower concentrations due to stock concentration limitations. Testing was performed with the organisms alone or with two *C. difficile* isolates present individually at 3 x Limit of Detection (LOD) of the cobas Cdiff Test. Results indicated that none of these organisms interfered with detection of the intended *C. difficile* targets. None produced false positive results when there was no intended *C. difficile* target present.

Clostridium botulinum cross reactivity was evaluated *in silico* using BLAST and Fuzznuc programs against the GenBank nucleotide sequence database to mimic PCR amplicon generation and probe detection steps. *In silico* analysis indicated that cross reactivity with *C. botulinum* is highly unlikely.

Table 9. Microorganisms and human cells tested

<i>Abiotrophia defectiva</i>	<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
<i>Aeromonas hydrophila</i>	<i>Alcaligenes faecalis</i> subsp. <i>Faecalis</i>	<i>Anaerococcus tetradius</i>
<i>Bacillus cereus</i>	<i>Bacillus cereus</i>	<i>Bacteroides caccae</i>
<i>Bacteroides merdae</i>	<i>Bacteroides stercoris</i>	<i>Bifidobacterium adolescentis</i>
<i>Bifidobacterium longum</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>
<i>Candida albicans</i>	<i>Candida catenulata</i>	<i>Cedecea davisae</i>
<i>Chlamydia Trachomatis</i> Serovar L2	<i>Citrobacter amalonaticus</i>	<i>Citrobacter freundii</i>
<i>Citrobacter koseri</i>	<i>Citrobacter sedlakii</i>	<i>Collinsella aerofaciens</i>
<i>Corynebacterium genitalium</i>	<i>Desulfovibrio piger</i>	<i>Edwardsiella tarda</i>
<i>Eggerthella lenta</i>	<i>Enterobacter aerogenes</i>	<i>Enterobacter cloacae</i>
<i>Enterococcus casseliflavus</i>	<i>Enterococcus cecorum</i>	<i>Enterococcus dispar</i>
<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>	<i>Enterococcus gallinarum</i>
<i>Enterococcus hirae</i>	<i>Enterococcus raffinosus</i>	<i>Escherichia coli</i>
<i>Escherichia coli</i>	<i>Escherichia fergusonii</i>	<i>Escherichia hermannii</i>
<i>Fusobacterium varium</i>	<i>Gardnerella vaginalis</i>	<i>Gemella morbillorum</i>
<i>Hafnia alvei</i>	HCT-15 Human Cells	<i>Helicobacter fennelliae</i>
<i>Helicobacter pylori</i>	<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>
<i>Lactobacillus acidophilus</i>	<i>Lactobacillus reuteri</i>	<i>Lactococcus lactis</i>
<i>Leminorella grimontii</i>	<i>Listeria grayi</i>	<i>Listeria innocua</i>
<i>Listeria monocytogenes</i>	<i>Mitsuokella multacida</i>	<i>Mobiluncus curtisii</i>
<i>Moellerella wisconsensis</i>	<i>Morganella morganii</i>	<i>Neisseria gonorrhoeae</i>
<i>Peptoniphilus asaccharolyticus</i>	<i>Peptostreptococcus anaerobius</i>	<i>Plesiomonas shigelloides</i>
<i>Porphyromonas asaccharolytica</i>	<i>Prevotella melaninogenica</i>	<i>Proteus mirabilis</i>
<i>Proteus penneri</i>	<i>Providencia alcalifaciens</i>	<i>Providencia rettgeri</i>
<i>Providencia stuartii</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas putida</i>
<i>Ruminococcus bromii</i>	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i>	<i>Salmonella enterica</i> subsp. <i>arizonae</i> (f.k.a. <i>Salmonella choleraesuis</i> ssp. <i>arizonae</i>)
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Choleraesuis</i>	<i>Serratia liquefaciens</i>	<i>Serratia marcescens</i>
<i>Shigella boydii</i>	<i>Shigella dysenteriae</i>	<i>Shigella sonnei</i>
<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Stenotrophomonas maltophilia</i>
<i>Streptococcus agalactiae</i>	<i>Streptococcus dysgalactiae</i>	<i>Streptococcus intermedius</i>
<i>Streptococcus uberis</i>	<i>Trabulsiella guamensis</i>	<i>Veillonella parvula</i>
<i>Vibrio cholerae</i>	<i>Vibrio parahaemolyticus</i>	<i>Yersinia bercovieri</i>
<i>Yersinia rohdei</i>	Cytomegalovirus (HHV5)*	Human Adenovirus 40*
Human Coxsackievirus A 10	Human Echovirus 11	Human Enterovirus 71
Human Rotavirus*	Norovirus GII	-

* Cytomegalovirus (HHV5) at 2.0×10^3 IU/mL, Human Adenovirus Type 40 was spiked at 2.2×10^3 PFU/mL, and Human Rotavirus at 9.8×10^3 PFU/mL for testing.

Table 10. *Clostridium* genus organisms tested, including non-toxigenic *C. difficile*

<i>Clostridium beijerinckii</i>	<i>Clostridium bifermentans</i>	<i>Clostridium bolteae</i>
<i>Clostridium botulinum</i> *	<i>Clostridium butyricum</i>	<i>Clostridium chauvoei</i>
<i>Clostridium difficile</i> Serogroup B (non-toxigenic)	<i>Clostridium difficile</i> Serogroup I (non-toxigenic)	<i>Clostridium fallax</i>
<i>Clostridium haemolyticum</i>	<i>Clostridium histolyticum</i>	<i>Clostridium innocuum</i>
<i>Clostridium methylpentosum</i>	<i>Clostridium nexile</i>	<i>Clostridium novyi</i>
<i>Clostridium orbiscindens</i> (re-named <i>Flavonifractor plautii</i>)	<i>Clostridium paraputrificum</i>	<i>Clostridium perfringens</i>
<i>Clostridium ramosum</i>	<i>Clostridium scindens</i>	<i>Clostridium septicum</i>
<i>Clostridium sordellii</i>	<i>Clostridium sphenoides</i>	<i>Clostridium spiroforme</i>
<i>Clostridium sporogenes</i>	<i>Clostridium symbiosum</i>	<i>Clostridium tertium</i>
<i>Clostridium tetani</i>	-	-

* Based on *in silico* analysis

Interference

Twenty-six commonly used medications, as well as fecal fat, whole blood, and mucin, were tested for potential interference effects with the cobas Cdiff Test. The amount of potentially interfering substance is expressed as the concentration in the primary stool specimen. The effects of the potentially interfering substances were evaluated in the presence and absence of two toxigenic *C. difficile* isolates spiked to approximately three times the LOD of the cobas Cdiff Test as determined for those strains in the LoD study (54 and 113 CFU/mL). No interference was observed for the exogenous substances tested. For fecal fat, no interference was observed up to 28%, for whole blood, no interference was observed up to 50%, and for mucin, no interference was observed up to 25%. Mucin at 50% interfered with the detection of the toxigenic *C. difficile* isolates. These results are summarized in Table 11.

Table 11. Potentially interfering substances tested

Substance	Concentration	Results
Fecal Fat	4 ~ 28 % (w/v)	No interference
Whole blood	25, 50 % (v/v)	No interference
Mucin	25, 50* % (w/v)	No interference up to 25% (w/v)
Tums	10% (w/v)	No interference
Vancomycin	1% (w/v)	No interference
Metronidazole	10% (w/v)	No interference
Imodium AD [®]	10% (w/v)	No interference
Stool Softener	10% (w/v)	No interference
Pepto-Bismol [®] (Procter & Gamble)	10% (v/v)	No interference

Substance	Concentration	Results
Nystatin Ointment USP	10% (w/v)	No interference
Preparation H [®] with Bio-Dyne [®] Cream (Wyeth)	10% (w/v)	No interference
GYNOL II	10% (w/v)	No interference
Vagisil [®] Anti-itch cream	10% (w/v)	No interference
Anusol [®] Plus	10% (w/v)	No interference
Sunscreen	1% (w/v)	No interference
Monistat [®] 7	10% (w/v)	No interference
Vaseline [™]	10% (w/v)	No interference
SAB-Dimethyldiolate [®] Suppositories (SABEX [®])	10% (w/v)	No interference
Mineral Oil	10% (v/v)	No interference
Equate Natural Vegetable Laxative	10% (w/v)	No interference
Dulcolax [®]	10% (w/v)	No interference
Fleet [®] (CB Fleet Company)	10% (w/v)	No interference
K-Y Jelly/Gelée [®] (McNeil-PPC)	1% (w/v)	No interference
Afrin Original Nasal Spray	10% (v/v)	No interference
Witch hazel	Liquid from 1 wipe/swab	No interference
E-Z-HD [™] High Density Barium Sulfate for suspension (E-Z-EM Canada)	20% (w/v)	No interference
Palmitic acid	10% (w/v)	No interference
Stearic acid	10% (w/v)	No interference
Aleve	10% (w/v)	No interference

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

Cross Contamination and Carryover Contamination:

The cross contamination rate was assessed by testing a series of high positive toxigenic *C. difficile* samples and negative samples in a checkerboard configuration on the cobas 4800 system. High positive samples were prepared by spiking pooled negative stool matrix with *C. difficile* culture to generate a Ct of the 95th percentile of the clinical specimen population. A total of 94 samples were included per run.

A total of nine runs were performed using three cobas 4800 systems with alternating checkerboard configurations to assess the cross contamination rate. A total of three runs of negative samples following checkerboard runs were performed to assess the carry-over contamination rate. In the nine checkerboard runs, one out of 423 negative samples produced a positive result for a cross contamination rate of 0.24%. None of the 282 negative samples from the carry-over contamination runs produced a positive result.

f. Assay cut-off:

Data from pre-clinical testing of stool specimens as well as limit of detection, inclusivity, and within-laboratory repeatability studies were compiled and analyzed to verify the preliminary cut-off value. None of the negative results exhibited a Ct value before the cut-off. The cut-off was validated in the prospective clinical study which demonstrated an adequate separation between the cut-off and the highest Ct value observed for a positive specimen.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of the cobas Cdiff Test was established in an IRB-approved, prospective, multi-site investigation comparing the results with direct and enriched 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. 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 the recovered isolates tested positive by cytotoxicity testing. If toxigenic *C. difficile* was isolated from the direct culture, the enrichment culture was not further processed. 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. 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. Discrepant analysis was performed using an FDA-cleared nucleic acid amplification test (NAAT) on all samples with discordant results between the cobas Cdiff Test and toxigenic culture.

Specimens were collected from 683 evaluable 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 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 of direct and enrichment toxigenic culture, for a prevalence rate of 20.7% for the study.

In a total of 49 cobas Cdiff Test runs in the clinical study, all external positive and negative controls were valid. Two failed runs (4.1%) were observed in the study due to one hardware error and one operational incident. Out of 683 specimens, the initial cobas Cdiff Test invalid rate was 0.3% (2/683) and the final invalid rate following one retest as per the labelled instructions for use was 0% (0/683). The initial cobas Cdiff Test failed rate was 0.7% (5/683) and the final failed rate following one retest was 0% (0/683).

Comparison with combined direct and enrichment culture

The clinical performance of the cobas Cdiff Test compared with the combined direct culture and enrichment culture results is shown in Table 12. The sensitivity and specificity of the cobas Cdiff Test were 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 were 94.9% (95% CI: 89.9% to 97.5%) and 98.2% (95% CI: 96.6% to 99.0%), respectively.

Table 12. 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

^c Of the seven specimens with false-positive cobas Cdiff Test results relative to combined direct and enrichment culture, three were positive and four 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 Table 13. The PPA and NPA of the cobas Cdiff Test compared to the initial direct culture for all 683 subjects were 97.3% (110/113) and 94.9% (541/570), respectively. Of the three specimens with false-negative cobas Cdiff Test results relative to direct culture, all three were negative by a second NAAT method.

Table 13. 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 three specimens with false negative cobas Cdiff Test results relative to direct culture, all three 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 a second NAAT method, and one sample was not further tested because of insufficient specimen volume.

b. Clinical specificity:

See section M3a

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the prospective clinical study, specimens were collected from 683 subjects suspected of CDI, with 306 males (44.8%) and 377 females (55.2%) and a mean age of 56 years (range 3 to 99). The percentage of positive results observed with the cobas Cdiff Test in this population was 20.4% (139/683).

N. Instrument Name:

cobas 4800 System

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes X or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No X

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No _____

3. Specimen Identification:

Specimens are identified using barcodes

4. Specimen Sampling and Handling:

Specimens are placed on the cobas x 480 instrument as open tubes and specimen processing is fully automated. After completion of specimen processing, the user transfers the plate carrier to the cobas z 480 instrument for automated amplification and detection.

5. Calibration:

No calibration is required by the user. Roche technicians perform calibration periodically as required.

6. Quality Control:

Positive and Negative Controls are included in every run. An Internal control is introduced for each Control and Specimen during sample preparation for testing on the cobas x 480 instrument.

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

Q. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.