510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K171770

B. Purpose for Submission:

To obtain a substantial equivalence determination for the cobas Cdiff Nucleic acid test for use on the cobas Liat System

C. Measurand:

tcdB gene of toxigenic *Clostridium difficile*

D. Type of Test:

Real-time PCR assay

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

cobas Cdiff Nucleic acid test for use on the cobas Liat System

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

   II

3. Product code:

   OZN, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

   The assay has only been validated for use with unformed or partially formed stool specimens that have been transferred into the cobas PCR Media tube according to the Instructions For Use.

4. Special instrument requirements:

   cobas Liat Analyzer

I. Device Description:

The cobas Cdiff Nucleic acid test for use on the cobas Liat System (cobas Liat 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 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.

Reagents and controls:

- cobas Cdiff Assay Tube
- cobas Cdiff Positive and Negative Control Kit for use on the cobas Liat System

Additional materials required but not provided:

- cobas PCR Media Uni Swab Sample Kit

J. Substantial Equivalence Information:

1. Predicate device name(s):

   cobas Cdiff Test for use on the cobas 4800 System

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

   K142422

3. Comparison with predicate:

Similarities		
Item	Device **cobas Cdiff Nucleic acid test for use on the cobas Liat System**	Predicate **cobas Cdiff Test for use on the cobas 4800 System (K142422)**
Sample type	Unformed soft stool specimens	Same
Amplification Technology	Real-time PCR	Same
Detection Technology	TaqMan probes with fluorescent dyes	Same
Assay target	*C. difficile* Toxin B gene (*tcd*B)	Same
Sample extraction	Automated magnetic bead-based nucleic acid extraction	Same

Differences		
Item	Device **cobas Cdiff Nucleic acid test for use on the cobas Liat System**	Predicate **cobas Cdiff Test for use on the cobas 4800 System (K142422)**
Instrument platform	cobas Liat system	cobas 4800 system
Samples/controls per run	One sample per run	Up to 24 or 96 samples per run
Test duration	Results within ~20 minutes after specimen loading	Results within 2.5 hours after specimen loading
Internal control design	A whole organism, gram-positive bacterial control (chemically inactivated *Bacillus thuringiensis israelensis*)	Lambda phage with encapsulated internal control sequence

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

Not applicable

L. Test Principle:

The cobas Liat 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 a 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.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Reproducibility

   The reproducibility of the cobas Liat Cdiff test was established in a multi-site investigation (two external sites and one 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. Only those valid results were included in the final percent agreement analysis.

   Panels consisted of three members: one negative specimen and two specimens with

different concentrations of one strain of toxigenic C. *difficile:* a low positive concentration near the limit of detection (~1X LOD), and a moderate positive concentration (~3X LOD). A run was defined as testing of three replicates of a panel member. For each of three lots, panels were run on five different nonconsecutive days by two different operators at each of the three sites.

Table 1 shows the percent agreement to expected results by panel member. Results were in agreement when a positive panel member had a valid result of Positive (Cdiff Detected) for the analyte or when the negative panel member had a valid result of Negative (Cdiff Not Detected) for the analyte. For the ~1X LOD panel members, 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 ~3X LOD panel member and 100% for the negative panel member.

Table 1. Reproducibility - Qualitative Results

Panel Member	Number of Valid Test Results	Percent Agreement with Expected Result	(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

Table 2 below presents the overall standard deviation (SD) and percent coefficient of variation (% CV) of cycle threshold (Ct) values for positive panel members at ~1X LOD and ~3X 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 three 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 Ct value for all positive panel members. Within each component, the % CV was ≤ 1.6% across positive panel members.

Table 2. Reproducibility - Ct Value Results

			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%

b. Linearity/assay reportable range:

Not applicable

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

Internal Control
A whole organism Internal Control is included in the assay tube and automatically added to all samples at the start of sample preparation. The cobas Liat Cdiff Internal Control is a chemically-inactivated bacterium (*Bti*) that is included in each cobas Cdiff assay tube and processed along with each sample. The Internal Control checks for adequate processing of the target bacteria through all steps of the assay and monitors the presence of inhibitors in the sample preparation and PCR. The cobas Liat Cdiff Internal Control should be positive in a negative sample and can be negative or positive in a Cdiff positive sample.

C. difficile External Positive and Negative Controls
Positive Control
The cobas Liat Cdiff Positive Control contains non-infectious DNA plasmids with *C. difficile* target sequence. The cobas Liat Cdiff Positive Control verifies the integrity of reagents in the cobas Cdiff assay tube and proper function of the cobas Liat Analyzer.

Negative Control
The cobas Liat Negative Control contains no target and monitors potential target contamination in the workflow or environment.

One cobas Liat Cdiff Positive Control and one cobas Liat Negative Control are run during the "Add Lot" procedure described earlier. Valid results must be obtained for both the Positive and Negative Control for the new lot of cobas Cdiff assay tubes to be validated on the instrument. Additional control runs may be performed after the "Add Lot" procedure in accordance with local, state, federal and/or accrediting organization requirements.

Across all sites during the clinical study, a total of 387 QC external control batches (including both positive and negative controls) were run. Of these QC batches, 376 (97.2%) were valid, two (0.5%) failed due to hardware issues, and nine (2.3%) were invalid due to reagent or protocol issues.

Sample stability
Unformed stool sample stability was demonstrated by testing *C. difficile* positive and negative clinical specimens on the cobas Liat Cdiff test after storage at 15 or 30°C for two days, and also after storage at 2-8°C for an additional seven days. The study also evaluated the stability of stool samples re-suspended in cobas PCR media. The results supported the labeled claims of unformed stool sample stability at 2-30°C for two days, at 2-8°C for nine days, and the stability of stool sample resuspended in cobas PCR media for seven days at 2-30°C.

d. Detection limit:

Analytical sensitivity

The Limit of Detection (LoD) for the cobas Liat Cdiff test was determined by analyzing quantified *C. difficile* cultures diluted to multiple concentration levels in negative stool matrix. Two toxigenic *C. difficile* strains were tested: ATCC 43255(VPI 10463) and R12087 (CDI 196). Negative stool matrix samples were also run to verify the Limit of Blank (LoB). All *C. difficile* concentrations were tested in three replicates each using two unique lots of cobas Cdiff assay tubes. The lowest level with 100% hit rate was tested with additional replicates to confirm the LOD level. If the overall hit rate for that level was less than 95%, the panel level above was tested with additional replicates. The final LOD level was confirmed with at least 21 additional replicates. LOD for this test is defined as the target concentration which can be detected as positive in ≥ 95% of the replicates tested, based on results generated by the worst performing reagent lot. The results are shown in Table 3.

Table 3. Limit of Detection

Strain ID	Toxinotype	REA* Type	PFGE† 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 Electrophoresis

Inclusivity

The inclusivity of the cobas Liat Cdiff test was evaluated on 37 toxigenic strains representing additional toxinotypes. Three replicates per strain were tested at three times the LOD level (~60 CFU/ml equivalent to 270 CFU/swab) as determined for ATCC 43255. At this inoculum level, all 37 toxigenic strains were detected. The tested strains are listed in Table 4.

Table 4. Inclusivity Strains

Cdiff Strain	Toxinotype	Ribotype
RMSCC 11251 (ATCC#BAA-1382; 630)	0	012
EX 623	I	102
AC 008	II	103
RMSCC 12827 [2004118; CDC-204118 (NAP-1)]	III	027
SE 844	IIIa	080
CH6230	IIIc	N/A
RMSCC 11298 (P43)	IV	N/A

Cdiff Strain	Toxinotype	Ribotype
55767	IV	023
RMSCC 11300 (2748-06)	V	078
SE 881	V	045
RMSCC 11302 (SE 1203)	VI	033
57267	VII	063
RMSCC 12472 (ATCC# 43598; 1470)	VIII	017
RMSCC 11299 (51680)	IX	019
RMSCC 11304 (CCUG 8864/STCC20309)	X	036
RMSCC 11308 (F15)	XII	N/A
IS 25	XII	056
R 9367	XIII	070
R 10870	XIV (New-XIVa)	111
R 9385	XV (New XIVb)	122
SUC36	XVI	078
RMSCC 11309 (No 1313)	XVII	232
K095	XVIII	014
TR13	XIX	N/A
TR14	XX	N/A
CH6223	XXI	N/A
CD07-468	XXII	N/A
8785	XXIII (New-IXc)	N/A
597B	XXIV	131
7325	XXV	027
7459	XXVI	N/A
KK2443/2006	XXVII	N/A
CD08-070	XXVIII	126
CD07-140	XXIX	056
ES 130	XXX	N/A
WA 151	XXXI	N/A
173070	XXXII	N/A

e. *Analytical specificity:*

Cross reactivity/ Microbial interference
To assess the analytical specificity of the cobas Liat Cdiff test, a panel was tested that included 117 bacteria, fungi and viruses that may be found in stool specimens, one

type of human cell, and 29 *Clostridium* genus organisms, including non-toxigenic *C. difficile* (Table 5 and Table 6). No wet testing was conducted for *Clostridium botulinum;* analytical specificity for this organism was predicted using BLAST program analysis against the GenBank nucleotide sequence database to mimic PCR amplicon generation.

All bacteria and human cells were spiked to 1 x 10^6 Units/mL, and all viruses were spiked to 1 x 10^5 Units/mL equivalent in stool matrix. Bacteria were quantified in colony forming units (CFU)/mL, human cells were quantified in cells/mL, and viruses were quantified in $TCID_{50}$/mL, except for *Chlamydia trachomatis*, which was quantified in IFU/mL.

The organisms were tested alone or in the presence of two toxigenic *C. difficile* isolates spiked at 3X LOD of the cobas Liat Cdiff test. Results indicated that none of the test organisms interfered with detection of intended toxigenic *C. difficile* targets. None of the test organisms produced false positive results in the absence of toxigenic *C. difficile*.

Table 5. Cross Reactivity Panel – Non-*Clostridium* Organisms and Human Cells

Abiotrophia defectiva	*Acinetobacter baumannii*	*Acinetobacter lwoffii*
Aeromonas hydrophila	*Alcaligenes faecalis* ATCC 35655	*Alcaligenes faecalis* subsp. *faecalis* ATCC 15554
Alcaligenes faecalis subsp. *faecalis* ATCC 8750	*Anaerococcus tetradius*	*Bacillus cereus* ATCC 11778
Bacillus cereus ATCC 13472	*Bacteroides caccae*	Bacteroides fragilis
Bacteroides merdae	*Bacteroides stercoris*	*Bifidobacterium adolescentis*
Bifidobacterium longum	*Campylobacter coli ATCC 33559*	*Campylobacter jejuni ATCC 43479*
Campylobacter jejuni Subsp. jejuni ATCC 33292	*Candida albicans*	*Candida catenulata*
Cedecea davisae	*Chlamydia Trachomatis Serovar L2 LGVII454*	*Citrobacter amalonaticus*
Citrobacter freundii	*Citrobacter koseri*	*Citrobacter sedlakii*
Collinsella aerofaciens	*Corynebacterium genitalium*	*Desulfovibrio piger*
Edwardsiella tarda	*Eggerthella lenta*	*Enterobacter aerogenes*
Enterobacter cloacae	*Enterococcus casseliflavus*	*Enterococcus cecorum*
Enterococcus dispar	*Enterococcus faecium van A*	*Enterococcus faecalis Van B*
Enterococcus gallinarum van C	*Enterococcus hirae*	*Enterococcus raffinosus*
Escherichia coli ATCC 11775	*Escherichia coli* ATCC 25922	*Escherichia coli O157:H7* ATCC 700927
Escherichia fergusonii	*Escherichia hermannii*	*Fusobacterium varium*
Gardnerella vaginalis	*Gemella morbillorum*	*Hafnia alvei*
HCT-15 Human Cells	*Helicobacter fennelliae*	*Helicobacter pylori*
Klebsiella oxytoca	*Klebsiella pneumoniae subsp. pneumoniae*	*Lactobacillus acidophilus*
Lactobacillus reuteri	*Lactococcus lactis*	*Leminorella grimontii*
Listeria grayi	*Listeria innocua*	*Listeria monocytogenes* ATCC 15313
Listeria monocytogenes ATCC BAA-839	*Mitsuokella multacida*	*Mobiluncus curtisii*

Moellerella wisconsensis	*Morganella morganii*	*Neisseria gonorrhoeae*
Peptoniphilus asaccharolyticus	*Peptostreptococcus anaerobius*	*Plesiomonas shigelloides*
Porphyromonas asaccharolytica	*Prevotella melaninogenica*	*Proteus mirabilis* ATCC 25933
Proteus mirabilis ATCC 29906	*Proteus penneri*	*Providencia alcalifaciens*
Providencia rettgeri	*Providencia stuartii*	*Pseudomonas aeruginosa* ATCC 35554
Pseudomonas aeruginosa ATCC 33584	*Pseudomonas putida*	*Ruminococcus bromii*
Salmonella enterica serovar Choleraesuis ATCC 7001	*Salmonella enterica subsp. Arizonae* ATCC 13314 *(f.k.a. Salmonella choleraesuis subsp. arizonae)*	*Salmonella enterica subsp. enterica* CMCC 1975
Salmonella enterica subsp. enterica serovar Typhi ATCC 19430	*Salmonella enterica subsp.enterica serovar Typhimurium* ATCC 14028	*Serratia liquefaciens* CMCC 169
Serratia liquefaciens ATCC 27592	*Serratia marcescens* ATCC 13880	*Serratia marcescens* ATCC 8100
Shigella boydii	*Shigella dysenteriae*	*Shigella sonnei*
Staphylococcus aureus	*Staphylococcus epidermidis*	*Stenotrophomonas maltophilia*
Streptococcus agalactiae	*Streptococcus dysgalactiae*	*Streptococcus intermedius*
Streptococcus sp.strain V8 ATCC 12973	*Streptococcus uberis*	*Trabulsiella guamensis*
Veillonella parvula	*Vibrio cholerae*	*Vibrio parahaemolyticus*
Yersinia bercovieri	*Yersinia rohdei*	Cytomegalovirus (HHV5)
Human Adenovirus Type 41	Human Coxsackievirus A4	Human Coxsackievirus B4
Human Echovirus 11	Human Enterovirus 71	Human Rotavirus
Norovirus GII	-	-

Table 6. Cross ReactiviTable 7. Cross Reactivity Panel - *Clostridium* organisms

Clostridium beijerinckii	*Clostridium bifermentans*	*Clostridium bolteae*
*Clostridium botulinum**	*Clostridium butyricum*	*Clostridium chauvoei*
Clostridium difficile Serogroup B (non-toxigenic)	*Clostridium difficile Serogroup I (non-toxigenic)*	*Clostridium fallax*
Clostridium haemolyticum	*Clostridium histolyticum*	*Clostridium innocuum*
Clostridium methylpentosum	*Clostridium nexile*	*Clostridium novyi*
Clostridium orbiscindens (renamed Flavonifractor plautii)	*Clostridium paraputrificum*	*Clostridium perfringens*
Clostridium ramosum	*Clostridium scindens*	*Clostridium septicum*
Clostridium sordellii	*Clostridium sphenoides*	*Clostridium spiroforme*
Clostridium sporogenes ATCC 15579	*Clostridium sporogenes CCRI 11128*	*Clostridium symbiosum*
Clostridium tertium	*Clostridium tetani*	-

*Results predicted from BLAST program analysis

Interference

Thirty eight commonly used medications, as well as fecal fat, whole blood, and mucin, were tested for potential interference effects with the cobas Liat 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 the concentration spiked in the primary stool specimen in Table 8. Two *C. difficile* isolates were spiked to 3X LOD of the cobas Liat Cdiff test and used as

targets in the tests.

Exogenous substances at the highest tolerable concentration with no interference on cobas Liat Cdiff test are shown in Table 8. Exogenous substances concentrations higher than listed in Table 8 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% capacity of the transfer swab.

Table 8. Interference Panel

Substance	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)
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)#

Substance	Concentration
Vancomycin	100% (w/v)
Vaseline	100% (w/v)
Sun Screen	100% (w/v)
Monistat Vaginal Insert	100% (w/v)
Vaginal Contraceptive Film	1 film per 20mL stool sample
Spermicidal Condoms	1 condom per 20mL stool sample

Specimens containing these substances at higher concentrations may generate invalid results.
* Specimens containing these substances at higher concentrations may generate false negative results.

f. Assay cut-off:

Data from pre-clinical testing of stool specimens were compiled and analyzed to verify the preliminary cut-off value. The cut-off was validated in the prospective clinical study which demonstrated an adequate separation of the negative and positive sample results in the study.

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 Liat Cdiff test was established in a prospective, multi-site investigation in comparison to the combined results of direct and broth enriched toxigenic culture using leftover, de-identified, unformed stool samples from patients suspected of having *C. difficile* infection.

Nine study sites from geographically diverse locations participated in this study. Nineteen operators distributed across these study sites performed testing with the cobas Liat Cdiff test. Reference culture was performed at a reference laboratory. Discrepant analysis testing was conducted using an FDA-cleared comparator NAAT.

A total of 1188 fresh remnant specimens were prospectively collected from symptomatic patients suspected of CDI. Of these, 172 were excluded according to the criteria for enrollment, storage, or cobas Liat Cdiff test processing as defined in the protocol. Of the 1,016 specimens tested with the cobas Liat Cdiff test, 1.4%

(14/1016) were initially invalid and 0.2% (2/1016) initially had failed results. After one retest per invalid or failed result following the labeled instructions, the final invalid rate was 0.1% (1/1016) and the final failed rate was 0% (0/1016). The three repeat invalid test results were excluded from the final performance analysis.

A total of 1,013 evaluable specimens were included in the study from 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 Liat 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 of 17.7% for the study.

Comparison with Combined Direct and Broth Enrichment Culture
The clinical performance of the cobas Liat Cdiff test compared with the combined results of direct and enrichment toxigenic culture are shown in Table 9. The sensitivity and specificity of the cobas Liat 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 Liat Cdiff test results relative to combined direct culture and enrichment culture, 19 were negative, three were positive and one was invalid by an FDA-cleared NAAT for *tcdB* DNA. Of the 16 specimens with false-positive cobas Liat Cdiff test results relative to combined direct and enrichment culture, 14 were positive and two were negative by the FDA-cleared NAAT.

Table 9. Comparison with Combined Direct and Enriched Culture

		Combined Direct and Enrichment Culture Result		
		Positive	**Negative**	**Total**
cobas Liat Cdiff Test Result	**Detected**	156	16[a]	172
	Not Detected	23[b]	818	841
	Total	179	834	1013

			95% CI
Sensitivity	87.2%	(156/179)	81.5% to 91.3%
Specificity	98.1%	(818/834)	96.9% to 98.8%
PPV	90.7%	(156/172)	85.4% to 94.2%
NPV	97.3%	(818/841)	95.9% to 98.2%

[a] Of the 16 specimens with false positive cobas Liat Cdiff test results relative to composite reference culture, 14 were positive by an FDA-cleared NAAT for *tcdB* DNA.
[b] Of the 23 specimens with false negative cobas Liat Cdiff test results relative to composite reference culture, 19 were negative, three were positive, and one had an invalid test result by an FDA-cleared NAAT for *tcdB* DNA.

Comparison with Direct Culture

The performance of the cobas Liat Cdiff test compared to direct culture is shown in Table. The positive percent agreement (PPA) and negative percent agreement (NPA) of the cobas Liat 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 eight specimens with false-negative cobas Liat Cdiff test results relative to direct culture, seven were negative and one was positive by a second NAAT method. Of the 33 specimens with false-positive cobas Liat Cdiff test results relative to direct culture, only 17 were tested with the second NAAT method: 14 were positive and three were negative by that second NAAT method. The remaining 16/33 specimens were positive by enriched culture and hence not tested with the second NAAT method per the discrepant analysis protocol.

Table 10. Comparison with Direct Culture

		Direct Culture Result		
		Positive	**Negative**	**Total**
cobas Liat Cdiff Test Result	**Detected**	139	33	172
	Not Detected	8	833	841
	Total	147	866	1013

			95% CI
PPA	94.6%	(139/147)	89.6% to 97.2%
NPA	96.2%	(833/866)	94.7% to 97.3%

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, 1013 samples were collected from a population that was 47.7% male (n=483) and 52.3% female (n=530) with a mean age of 57 years. The percentage of positive results observed with the cobas Liat Cdiff test in this population was 17%.

N. Instrument Name:

cobas Liat 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:
   A sample barcode is scanned or a sample ID entered into the cobas Liat System during the assay run process. After scanning the sample barcode, the corresponding sample is loaded directly into a cobas Liat Tube using a transfer pipette. After the tube is capped, the tube barcodes is scanned by the cobas Liat System and the tube is inserted into the system to start the test.

4. Specimen Sampling and Handling:
   Specimen sampling and handling during the assay is controlled automatically using multiple sample processing modules contained within the cobas Liat System. The sample processing modules are composed of two assemblies, a moving side assembly comprised of multiple sample processing plungers and clamps and a fixed side assembly. When performing an assay, a cobas Liat Tube is inserted into the tube slot of a cobas Liat System. The plungers and clamps selectively compress the cobas Liat Tube segments against the fixed side assembly to release reagents from the segments, move the sample from one segment to another, and control reaction conditions.

5. Calibration:
   Not required. The cobas Liat Tube is single use and part of a closed system.

6. Quality Control:
   An internal process control used in conjunction with procedural checks monitors instrument functionality, performance, fluidics, and result determination based on a pre-defined decision algorithm.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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