510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K260045

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas Cdiff Nucleic acid test for use on the cobas Liat System (07454945190); cobas Cdiff Positive and Negative Control Kit for use on the cobas Liat System (07454970190)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OZN	Class II	21 CFR 866.3130 – Clostridium Difficile Toxin Gene Amplification Assay	MI - Microbiology
OOI	Class II	21 CFR 862.2570 – Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To demonstrate that the *Cobas Cdiff Nucleic acid Test* run on a modified cobas Liat System with software version 4.0 is substantially equivalent to the previously cleared predicate device (i.e., K212427)

The updated cobas liat Analyzer Software (v4.0) incorporates the following changes:

(a) *Assay-related Changes*:(i) update of cobas Cdiff assay-specific script from the legacy "CDFA v1.1.0" to "CDFA v1.4.0 *generic Liat Assay Specific Packages* (gLASP) assay script" and (ii) replacement of the legacy result calculation algorithm with a new Roche Kinetic Algorithm v2 ("KA2," incorporated in the updated cobas Cdiff assay script).

(b) *Analyzer instrument-related Changes*: (i) OS upgrade and (ii) enablement of optional wireless network connectivity.

B Measurand:

Toxin B (*tcdB*) gene of toxigenic *Clostridioides difficile* (*C. difficile*)

C Type of Test:
Real-time PCR assay for qualitative detection of *C. difficile tcdB* gene in unformed (liquid or soft) stool specimens

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The cobas Cdiff Nucleic acid test for use on the cobas Liat System is an automated, qualitative *in vitro* diagnostic test, that uses real-time polymerase chain reaction (PCR), for the detection of the toxin B (*tcdB*) gene of toxigenic *Clostridioides difficile* (*C. 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.

C Special Conditions for Use Statement(s):
Rx – For Prescription Use Only

D Special Instrument Requirements:
cobas Liat Analyzer with cobas Liat Software v4.0

IV Device/System Characteristics:

A Device Description:
The cobas Cdiff Nucleic Acid test is a rapid, automated *in vitro* diagnostic (IVD) test for the qualitative detection and differentiation of *C. difficile* DNA in human stool specimens. The test is performed on the cobas Liat System which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using TaqMan probe-based real-time PCR assays. The system consists of an instrument and preloaded software for running tests and viewing the results. The system requires the use of a single-use disposable cobas Cdiff Nucleic Acid assay tube that holds all the sample purification and PCR reagents and hosts the sample preparation and PCR processes.

B Principle of Operation:
The cobas Cdiff assay targets the oligonucleotide sequence of the *tcdB* (i.e., Toxin B gene of toxigenic *C. difficile*) that is implicated in *C. difficile* pathogenesis and allows differentiation between toxigenic and non-toxigenic *C. difficile* strains. The assay device includes a gram-positive bacterium (namely, *Bacillus thuringiensis israelensis*) which is co-processed with the target during the assay to extract nucleic acids and amplify oligonucleotide sequences for use as an Internal Control.

The test runs on the cobas Liat System, which consists of the cobas Liat Analyzer (hardware with integrated software) and single-use disposable assay tubes that contain all necessary sample preparation and PCR reagents in separate segments divided by frangible seals. The system fully automates all nucleic acid amplification test processes, including reagent preparation, target

enrichment, inhibitor removal, nucleic acid extraction, amplification, real-time detection, and result interpretation.

C Instrument Description Information:

1. Instrument Name:
   cobas Liat Analyzer with cobas Liat Software v4.0

2. Specimen Identification:
   The cobas Liat system maintains traceability of specimen identification during processing and analysis by means of barcode labels present on each cobas Cdiff assay tube. Before starting the assay, the user is required to scan the assay tube barcode using the barcode reader integrated into the liat analyzer instrument. The specimen barcode is next scanned in, or a specimen identifier may be manually entered into the Liat system. The user adds the unformed (liquid or soft) stool specimen to the cobas Cdiff assay tube using a manufacturer-provided transfer pipette before closing the tube and re-scanning the cobas Cdiff assay tube barcode. The user next inserts the assay tube into the Liat analyzer and starts the run.

3. Specimen Sampling and Handling:
   As indicated in the package insert, unformed (liquid or soft) stool specimens from patients with suspected *C. difficile* infection are collected in a clean, dry, and unused container by following institutional standard operating procedures. Prior to testing, each stool specimen is transferred to individual cobas PCR Media tubes using swabs provided in cobas PCR Media Uni Swab Sample kits. The cobas PCR Media tube inoculated with the swab is vortexed, and an aliquot of each stool specimen suspension is transferred to individual single-use disposable cobas Cdiff assay tubes using a manufacturer-provided transfer pipette and placed in the cobas Liat analyzer.

4. Calibration:
   The Cobas Liat Analyzer hardware periodically performs automatic recalibration, and the Cobas Liat System does not run the assay until this auto-calibration step is performed.

5. Quality Control:
   a. ***Internal Control:*** The assay tube contains a whole organism Internal Control (*Bacillus thuringiensis israelensis* ("Bti"), a chemically inactivated gram-positive bacterium), which is automatically added to all samples at the start of sample preparation for co-processing along with each sample. The cobas Liat Cdiff 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 Internal Control is expected to be positive in a negative sample and can be negative or positive in a Cdiff positive sample. An invalid result will be generated if the Internal Control fails to meet the acceptance criteria.
   b. ***Per lot Quality Control (QC) procedure:*** As indicated in the package, insert, the user is instructed to run a QC procedure (called the "Add Lot" procedure) before using a new lot of cobas Cdiff assay tubes. This procedure is required to validate the lot of cobas Cdiff assay tubes to be used and involves running a Cobas Liat Negative Control sample and a Cobas Liat Cdiff Positive Control sample. Valid results must be obtained for both Positive and Negative Control for the new lot of Cobas Cdiff assay tubes to be validated on the instrument.

c. ***Cobas Cdiff Positive Control:*** The assay Positive Control contains non-infectious DNA plasmids with *C. difficile* target sequence in buffer and is intended to verify the integrity of reagents in the Cobas Cdiff assay tube and proper function of the Cobas Liat Analyzer.
d. ***Cobas Liat Negative Control:*** The assay Negative Control contains no target and is intended to monitor potential target contamination in the workflow or environment.

V Substantial Equivalence Information:

A Predicate Device Name(s):
cobas Cdiff nucleic acid test for use on the cobas Liat System

B Predicate 510(k) Number(s):
K212427

C Comparison with Predicate(s):

Candidate device & Predicate device:	Candidate: K260045	Predicate: K212427
Device Trade Name	Cobas Cdiff Nucleic Acid test for use on the cobas Liat System	-same-
General Device Characteristic Similarities		
Intended Use / 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 uses real-time polymerase chain reaction (PCR) for the detection of the toxin B (*tcdB*) gene of toxigenic *Clostridioides difficile* (*C. 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.	-same-
Conditions for use	For Prescription Use only	-same-
Regulation Number	21 CFR 866.3130; 21 CFR 862.2570	-same-
Classification	Clostridium difficile Toxin Gene Amplification Assay Real-Time Nucleic Acid Amplification System	-same-
Product Code	OZN, OOI	-same-
Sample Type	Unformed soft stool specimens	-same-
Amplification Technology	Real-time PCR	-same-
Internal Control	A gram-positive *Bacillus thuringiensis israelensis* bacterial organism to monitor the full process of Cobas Liat Analyzer. Native sequence in the bacteria is used as the Internal Control Target.	-same-
Positive Control	Plasmid in buffer	-same-
Negative Control	Buffer only	-same-

Candidate device & Predicate device:	Candidate: K260045	Predicate: K212427
Target Analyte	*C. difficile* Toxin B (*tcdB*) gene	-same-
Sample Collection Devices	Cobas PCR Media Swab Sample Kit	-same-
Sample Preparation	Magnetic bead-based nucleic acid extraction automated by the Cobas Liat Analyzer	-same-
Result Analysis	Based on PCR cycle threshold analysis	-same-
Assay Instrument	Cobas Liat Analyzer	-same-
General Device Characteristic Differences		
System software and assay algorithm	▪ Cobas Liat analyzer v4.0.0 ▪ Roche Kinetic Algorithm v2 (KA2) ▪ Generic Liat Assay Specific Packages (gLASP) assay script, CDFA v1.4.0	▪ Cobas Liat analyzer v3.3 ▪ Legacy algorithm ▪ Legacy IQuum Command Language (ICL) assay script, CFDA v1.1.0
Wireless connectivity	▪ Wired Ethernet connection ▪ Optional/alternative Wireless connectivity enabled via Off-The-Shelf USB Wi-Fi adapter	Wired Ethernet connection

VI Standards/Guidance Documents Referenced:

Class II Special Controls per 21 CFR §866.3130 *Class II Special Controls Guideline Document: Toxin Gene Amplification Assays for the Detection of Clostridium difficile | FDA*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

The analytical performance of the Cdiff Nucleic Acid Test was evaluated following an update to the assay script (from the Legacy IQuum Command Language (ICL) assay script CDFA v1.1.0 to the generic Liat Assay Specific Package (gLASP) assay script CDFA v1.4.0). The updated script incorporates Roche's standardized Kinetic Algorithm 2 (KA2). To assess the impact of these changes, all analytical data previously submitted in support of the cleared cobas Liat Nucleic Acid Test device were reprocessed using the KA2 algorithm.

1. Precision/Reproducibility:
   In support of the candidate device with updated assay script and algorithm, raw fluorescence data from a within-laboratory precision study and a clinical reproducibility study (originally conducted in support of K171770 with legacy algorithm) were reprocessed using the updated algorithm. The re-analysis confirmed that the analytical precision remained consistent with the performance precision claims currently described in the package insert.

2. Linearity:
   Not applicable.

3. Analytical Specificity/Cross-reactivity, Interference, and Inclusivity:
Previously obtained raw fluorescence data from analytical specificity and inclusivity studies originally conducted for K171770 were reprocessed using the updated algorithm for the current candidate device. The reanalysis confirmed that the outcomes of cross reactivity, interference (endogenous and exogenous), and inclusivity (for forty (40) toxigenic *C. difficile* strains spanning multiple toxinotypes) studies remained consistent with the performance claims as described in the Package Insert.

4. Detection Limit:
Previously obtained raw fluorescence data from a limit of detection (LoD) study originally conducted for K171770 was reprocessed using the updated algorithm for the current candidate device (K260045). The reanalysis confirmed that analytical sensitivity/LoD remained consistent with the performance claims as described in the Package Insert.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
Metrological traceability is not applicable since there is no international standard for the Cdiff Nucleic Acid test.

The updated KA2 algorithm introduces a systematic shift in Ct values; therefore, manufacturing release specifications were recalibrated to maintain lot-to-lot performance consistency for the candidate device. Reanalysis of raw fluorescence data from all previously obtained real-time stability data for cobas Cdiff assay tubes was conducted with the updated algorithm, and no changes to the previously established 15-month shelf-life were observed.

6. Assay Cutoff:
The Cdiff Nucleic acid assay PCR Ct cutoffs and QC release specifications were revised for the transition to the updated KA2 algorithm (which differs in computational metrics from the legacy algorithm). To confirm that the algorithm update did not affect assay performance characteristics or cleared claims, all previously submitted data (originally processed under legacy CDFA v1.1.0) were reprocessed with CDFA v1.4.0, and no appreciable changes were observed in the final assay results.

B Comparison Studies:

1. Method Comparison with Predicate Device:
To demonstrate performance equivalency of the Cdiff Nucleic acid assay following migration from the legacy assay script CDFA v1.1.0 running on software v3.5.1 to the updated assay script CDFA v1.4.0 running on software v4.0.0 and to verify all the changes to the cobas Liat system, a head-to-head equivalency study was conducted using ten (10) cobas Liat analyzer instruments, unique lots of positive and negative controls, as well as of swab kits and Cdiff assay tubes, and a contrived sample panel prepared with *C. difficile* ATCC 43255 at 2× and 5×LoD in cobas PCR media. In the study, the empaneled samples (along with media-only negatives) were tested on both the cleared and modified test systems. A total of 160 runs were performed (comprised of ten (10) replicates for each of negative and 5×LoD samples; forty (40) replicates at 2×LoD; and ten (10) replicates each of Positive and Negative controls samples, under each of the legacy and updated algorithms). All runs met the acceptance criteria and provided valid results, with no aborted or invalid runs. No deviations from the study protocol were observed.

The study concluded that migration from the legacy assay script to the updated gLASP assay script had no impact upon assay performance, confirming equivalency between the two script versions (as summarized in Table 1 below).

Table1: Equivalency study outcome for Cdiff target

Test sample	CDFA Script v1.1.0 (legacy)				CDFA Script v1.4.0 (gLASP)			
	Replicates	**Target detected**	**Target not detected**	**Hit rate**	**Replicates**	**Target detected**	**Target not detected**	**Hit rate**
Negative Control	10	0	10	0%	10	0	10	0%
Positive Control	10	10	0	100%	10	10	0	100%
Negative Sample	10	0	10	0%	10	0	10	0%
~2× LOD (positive) Sample	40	40	0	100%	40	40	0	100%
~5× LOD (positive) Sample	10	10	0	100%	10	10	0	100%

Footnotes:

1. Out of 160 runs, all runs gave valid results. There were no aborted or invalid runs.
2. There were no deviations observed from the predetermined Equivalency Study Protocol.
3. All acceptance criteria outlined in Equivalency Study Protocol for cobas Liat assay's migration to gLASP Architecture were met.

2. Matrix Comparison:
   Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:
   Raw fluorescence data from a previous Clinical Performance /Clinical Utility Study (conducted to support the original clearance of the cobas Liat Cdiff Nucleic acid test, K171770) were reprocessed under the updated algorithm to support K260045. The reanalysis demonstrated consistency in clinical performance between the legacy and updated algorithms, with continued fulfillment of primary acceptance criteria and absence of any significant change to performance claims in the Package Insert.

2. Clinical Specificity:
   Please refer to section C1 above.

3. Clinical Cut-Off
   Not Applicable.

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not Applicable.

D Expected Values/Reference Range:

Not Applicable.

E Other Supportive Instrument Performance Characteristics Data:

EMC: Electrical safety and electromagnetic compatibility (EMC) testing were performed; the information was reviewed and found to be acceptable

Software and Cybersecurity: Software and Cybersecurity documentations were reviewed and found to be acceptable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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