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

July 21, 2017

Luminex Corporation
Wendy Ricker
Manager, Regulatory Affairs
12212 Technology Blvd.
Austin, Texas 78727

Re: K171441
Trade/Device Name: ARIES C. difficile Assay Complete Kit, ARIES C. difficile Assay Protocol File Kit, ARIES C. difficile Assay Kit (24 cassettes), ARIES Stool Resuspension Kit
Regulation Number: 21 CFR 866.3130
Regulation Name: Clostridium difficile toxin gene amplification assay
Regulatory Class: Class II
Product Code: OZN, OOI
Dated: May 15, 2017
Received: May 16, 2017

Dear Wendy Ricker:

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 at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

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

Sincerely,

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)*
K171441

Device Name
ARIES *C difficile* Assay

Indications for Use *(Describe)*

The ARIES *C. difficile* Assay is a real-time polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection of toxigenic *Clostridium difficile* (*C. difficile*) nucleic acid in unpreserved, unformed (liquid or soft) stool specimens obtained from patients suspected of having *Clostridium difficile* infection (CDI). The test targets the *C. difficile* toxin A gene (*tcdA*) and toxin B gene (*tcdB*) and is indicated for use as an aid in the diagnosis of *C. difficile* infection (CDI).

The ARIES *C. difficile* Assay is indicated for use with ARIES Systems.

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

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

A. 510(k) Number:

K171441

B. Purpose for Submission:

Traditional 510(k), New Device

C. Measurand:

Targets DNA sequences of the toxin A (*tcdA*), and toxin B (*tcdB*) genes within the Pathogenicity Locus (PaLoc) of toxigenic strains of *Clostridium difficile*.

D. Type of Test:

Qualitative Real Time Polymerase Chain Reaction (PCR)

E. Applicant:

Wendy Ricker
Luminex Corporation
12212 Technology Blvd
Austin, TX 78727
Tel: (608) 203-8936

F. Proprietary and Established Names:

ARIES® *C. difficile* Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OZN	II	21 CFR 866.3130—*C. difficile* toxin gene amplification assay	Microbiology (83)

H. Intended Use:

1. Intended use(s):

The ARIES® *C. difficile* Assay is a real-time polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection of toxigenic *Clostridium difficile* (*C. difficile*) nucleic acid in unpreserved, unformed (liquid or soft) stool specimens obtained from patients suspected of having *Clostridium difficile* infection (CDI).

The test targets the *C. difficile* toxin A gene (*tcdA*) and toxin B gene (*tcdB*) and is indicated for use as an aid in the diagnosis of *C. difficile* infection (CDI).

The ARIES® *C. difficile* Assay is indicated for use with ARIES® Systems.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   For use with ARIES® Systems.

I. Device Description:

The ARIES® *C. difficile* Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system which consists of the ARIES® System or the ARIES® M1 System with their included ARIES® Software, a stool resuspension kit, an assay-specific cassette, and an assay-specific protocol file. The ARIES® *C. difficile* Assay cassette is a disposable, single-use cassette containing nucleic acid purification reagents, internal sample process control (SPC), and an assay-specific master mix capable of performing the designated assay on one sample. The ARIES® *C. difficile* Assay cassette directly detects toxigenic *Clostridium difficile (C. difficile*) from unformed stool specimens obtained from patients suspected of having *Clostridium difficile* infection. Specifically, the ARIES® *C. difficile* Assay cassette detects the *C. difficile* toxin A gene (*tcdA*) and toxin B gene (*tcdB*) and a DNA Sample Processing Control.

Unpreserved raw stool is processed using the ARIES® Stool Resuspension Kit. The ARIES® Stool Resuspension Kit includes a flocked swab, a tube containing preprocessing beads, and Stool Resuspension Buffer. The preprocessing method involves transfer of a swab of stool specimen into a tube containing preprocessing beads and Stool Resuspension Buffer. The swab is mixed in the tube by vortexing and then centrifuged. Finally, the preprocessed sample is added to the ARIES® *C. difficile* Assay cassette.

The specimen is lysed and nucleic acid is extracted using an ARIES® instrument. An extractable sample processing control (SPC) target is also present in the ARIES® *C. difficile* Assay cassette and is processed with the specimen. The SPC controls for recovery of extracted nucleic acid, for inhibitory substances and for PCR reagent and instrument integrity. The Ct value of the SPC is designed to verify nucleic acid extraction, to identify PCR inhibition, if any, and verify proper function of the extraction system and real-time instrument. The T_m value of the SPC is used as a reference for determining the T_m of the *tcdA* and *tcdB* targets (if present).

The extracted nucleic acid and SPC are transferred via magnetic beads through the cassette to the ARIES® *C. difficile* Assay lyophilized PCR reagents in the PCR tube that contain primer pairs specific to *tcdA*, *tcdB*, and the SPC sequence. Each of the primer pairs is labeled with a distinct fluorophore and detected in distinct channels of an ARIES® System. PCR amplification is performed and assay fluorescence is monitored. Incorporation of a quencher-labeled nucleotide results in a decrease in fluorescence for the associated primer pair. Following amplification, the reaction is slowly heated to separate the fluorescent-labeled strand from the quencher-labeled strand, a process that results in an increase in the fluorescence signal. The reaction fluorescence is measured during this process and the temperature at which the change in fluorescence is the maximum T_m of the amplicon. The strands of the amplicons will separate at a specific melting temperature (T_m) and an increase in fluorescence is observed. The instrument fluorescence output is analyzed and test results are determined using the ARIES® System software and the ARIES® *C. difficile* Assay protocol and run files. ARIES® *C. difficile* Assay results may be reported from the ARIES Software or from the optional SYNCT® Software.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Quidel Molecular Direct C. difficile Assay

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

   K123998

3. Comparison with predicate:

   The following tables compare Luminex's ARIES® *C. difficile* Assay to Quidel's Molecular Direct C. difficile Assay (k123998).

Table 11.1: Similarities between New Device and Predicate

Similarities		
Attribute	**New Device**	**Predicate Device (K123998)**
Intended Use	The ARIES® *C. difficile* Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection of toxigenic *Clostridium difficile* (*C. difficile*) nucleic acid in unpreserved, unformed (liquid or soft) stool specimens obtained from patients suspected of having *Clostridium difficile* infection (CDI). The test targets the *C. difficile* toxin A gene (*tcdA*) and toxin B gene (*tcdB*) and is indicated for use as an aid in the diagnosis of *C. difficile* infection (CDI). The ARIES® *C. difficile* Assay is indicated for use with ARIES® Systems.	The Quidel Molecular Direct *C. difficile* Assay is a qualitative, multiplexed *in vitro* diagnostic test for the direct detection of toxin A gene (*tcdA*) or toxin B gene (*tcdB*) sequences of toxigenic strains of *Clostridium difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having Clostridium difficile-Associated Disease (CDAD). The Quidel Molecular Direct C. difficile Assay is a real-time PCR test and utilizes proprietary sample preparation with fluorescently labeled primers and probes. The assay can be performed using either the Life Technologies QuantStudio® Dx; the Applied Biosystems 7500 Fast Dx, or the Cepheid SmartCycler II, to detect the toxin gene sequences associated with toxin-producing *C. difficile* strains. The assay is intended to be performed directly on CDAD-suspected stool specimens, and is indicated from use as an aid in the diagnosis of CDAD.
Analyte	Toxin A gene (*tcdA*) and Toxin B gene (*tcdB*)	Toxin A gene (*tcdA*) and Toxin B gene (*tcdB*)
Sample type	Unpreserved, unformed stool (liquid or soft)	Unpreserved, unformed stool (liquid or soft)
Assay format	Real-time PCR	Real-time PCR
Assay results	Qualitative	Qualitative
Automated	Yes	Yes

Table 11.2: Differences between New Device and Predicate

Differences		
Attribute	**New Device**	**Predicate Device (K123998)**
Detection method	Pairs of fluorescently-labeled primers with quencher labeled nucleotides. Measures decrease in assay fluorescence with each PCR cycle.	PCR with fluorescently labeled probes; detection based on Taqman chemistry. Measures an increase in assay fluorescence with each cycle.
Test Container	Disposable single-use cassette	Manual amplification set-up in PCR microfuge tubes or plates with wells
Instrument	ARIES® System, ARIES® M1 System	QuantStudio®Dx; the Applied Biosystems 7500 Fast Dx or the Cepheid SmartCycler II

K. Standards/Guidance Documents Referenced:

FDA Guidance Class II Special Controls Guideline Document: Toxin Gene Amplification Assays for the Detection of *Clostridium difficile*.

L. Test Principle:

The ARIES® *C. difficile* Assay chemistry is based on an expanded genetic alphabet technology, consisting of synthetic DNA base pair 2'-deoxy-5-methyl-isocytidine (iC): 2'-deoxyisoguanosine (iG). The isobases (iC and iG) pair specifically with each other and not with natural nucleotides. In addition, isobases are efficiently incorporated during PCR. During PCR amplification, a quencher-modified iGTP is incorporated by the polymerase opposite an iC and a fluorophore reporter attached to a PCR primer. If the target is present and is amplified, assay fluorescence decreases with every cycle as amplification product accumulates. The decrease in assay fluorescence is monitored in real time using an ARIES® System. Following PCR, the amplification products are thermally denatured and assay fluorescence is monitored. The strands of the amplification products are separated and assay fluorescence increases, thus enabling determination of the melting temperature (T_m) of the amplicon.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision

   Within Laboratory Precision/Repeatability of the ARIES® *C. difficile* Assay was evaluated by two operators performing testing across multiple ARIES® instruments using one lot of ARIES® *C. difficile* Assay Cassettes. Testing was performed on 12 days and included analysis of a total of 252 samples. A

repeatability panel was prepared, containing moderate positive, low positive and high negative/low positive samples, independently for two toxigenic *C. difficile* strains as well as a negative sample. The results of the repeatability study are presented in Table 11.3.

Table 11.3: ARIES® *C. difficile* Assay Within Laboratory Precision/Repeatability Results[a]

Target Type	Expected Positivity	Agreement with Expected	95% Confidence Interval
BAA-1870 Moderate Positive	100%	100% (36/36)	90.4 – 100%
BAA-1870 Low Positive	Approximately 95%	100% (36/36)	90.4 – 100%
BAA-1870 High Negative/Low Positive	20% – 80%	66.7% (24/36)	50.3 – 79.8%
BAA-1871 Moderate Positive	100%	100% (36/36)	90.4 – 100%
BAA-1871 Low Positive	Approximately 95%	97.2% (35/36) [b]	85.8 – 99.5%
BAA-1871 High Negative/Low Positive	20% – 80%	25.0% (9/36)	13.7-41.1%
***C. difficile* Negative**	100%	100% (36/36)	90.4% to 100%

[a] An overall invalid rate of 0.8% (2/254) was observed.
[b] 2/36 samples produced Invalid results on initial testing; both reported as Positive upon repeat

Reproducibility

Reproducibility of the ARIES® *C. difficile* Assay was evaluated by testing of one lot of ARIES® *C. difficile* Assay Cassettes on two ARIES® Instruments by two operators at each of three sites on 5 non-consecutive days. A reproducibility panel was prepared, containing moderate positive, low positive and high negative/low positive samples, independently for two toxigenic *C. difficile* strains as well as a negative sample. The reproducibility panels were created by an independent operator and blinded. The results of the reproducibility study are presented in Tables 11.4 and Table 11.5.

Table 11.4: ARIES® *C. difficile* Assay Site to Site Reproducibility Results[a]

	Site 1		Site 2		Site 3	
	Agreement with expected results		Agreement with expected results		Agreement with expected results	
BAA-1870 Moderate Positive	30/30	100%	30/30	100%	30/30	100%
BAA-1870 Low Positive	30/30	100%	30/30	100%	30/30	100%
BAA-1870 High Negative/Low Positive	28/30	93.3%	21/30	70%	27/30	90%
BAA-1871 Moderate Positive	30/30	100%	30/30	100%	30/30[b]	100%
BAA-1871 Low Positive	30/30	100%	30/30	100%	29/30	96.7%
BAA-1871 High Negative/Low Positive	23/30	76.7%	28/30	93.3%	24/30	80%
***C. difficile* Negative**	30/30	100%	29/30[b]	96.7%	30/30	100%

[a] The expected results for the reproducibility panel targets are 100% for Moderate Positive, 95% for Low Positive, and 20-80% for High Negative/Low Positive for BAA-1870 and BAA-1871. Negative target is expected to be 0% positive.
[b] 1/30 samples was reported as Invalid on initial testing; reported Toxigenic *C. difficile* Positive upon repeat

Table 11.5: Reproducibility Panel Total Results [a]

	Agreement with expected results		95% Confidence Interval
BAA-1870 Moderate Positive	90/90	100.0%	95.9 - 100.0%
BAA-1870 Low Positive	90/90	100.0%	95.9 - 100.0%
BAA-1870 High Negative/Low Positive	76/90	84.4%	75.6 - 90.5%
BAA-1871 Moderate Positive	90/90	100.0%	95.9 - 100.0%
BAA-1871 Low Positive	89/90	98.9%	94.0 - 99.8%
BAA-1871 High Negative/Low Positive	75/90	83.3%	74.3 - 89.6%
***C. difficile* Negative**	89/90[b]	98.9%	94.0 - 99.8%

[a] The expected results for the reproducibility panel targets are 100% for Moderate Positive, 95% for Low Positive, and 20-80% for High Negative/Low Positive for BAA-1870 and BAA-1871. Negative target is expected to be 0% positive.
[b] 1/90 replicates produced a false positive result. The SPC was observed to be positive in all replicates.

b. Linearity/assay reportable range:

Not applicable. The ARIES® *C. difficile* Assay is a qualitative assay.

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

Controls:

Process Control

Each ARIES® *C. difficile* Assay cassette contains a Sample Processing Control (SPC), which is processed with the sample and analyzed during the amplification reaction. The SPC verifies nucleic acid extraction, and proper reagent, cassette, ARIES® System, and assay protocol performance. The SPC has known melting temperature (T_m) and Ct ranges. Each time an assay is run, the system measures the melting temperature and fluorescence intensity of the SPC to ensure the thermal and optical subsystems have remained in calibration.

External Controls

External controls should be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference toxigenic *C. difficile* strain or well characterized toxigenic *C. difficile* clinical isolates may be used as positive controls. A non-toxigenic strain of *C. difficile* may be used as a Negative Control. Alternatively, clinical specimens known to be positive or negative for toxigenic *C. difficile* may be used as Positive and Negative External Controls, respectively. The ARIES® *C. difficile* Assay Cassette Kit does not include external positive and negative controls.

Stability:

Specimen Stability

Fresh specimen stability was determined for toxigenic *C. difficile* in a negative clinical stool pool at 2 – 8°C. This was assessed by testing six replicates of each of three *C. difficile* target concentrations and the negative clinical pool across five different time points. The *C. difficile* concentrations tested included a moderate positive, low positive and high negative/low positive. The results of the study demonstrated that specimens for the ARIES® *C. difficile* Assay are stable when stored at 2 – 8°C for up to 7 days.

Room temperature specimen stability was determined for toxigenic *C. difficile* in a negative clinical stool pool at 15 – 30°C. This was assessed by testing six replicates of each of three *C. difficile* target concentrations and the negative clinical stool pool across four different time points. The *C. difficile* concentrations tested included a moderate positive, a low positive, and a high negative/low positive. The results of the study demonstrated that specimens for the ARIES® *C. difficile* Assay are stable when stored at 15 – 30°C for up to 4 hours.

Frozen specimen stability was determined for toxigenic *C. difficile* in a negative clinical stool pool at -65 to -95°C. This was assessed by testing six replicates of each of three *C. difficile* target concentrations and the negative clinical stool pool across multiple time points. The *C. difficile* concentrations tested included a moderate positive, low positive and high negative/low positive. The results of

the study demonstrated that specimens for the ARIES® *C. difficile* Assay are stable when stored at -65 to -95°C for up to 3 months.

Freeze thaw specimen stability was determined for specimens after cycles of freezing and thawing. This was assessed by testing three *C. difficile* contrived target concentrations in negative stool matrix across five freeze thaw cycles. The concentrations used for testing were high positive, moderate positive, and low positive. The results of the study demonstrated that specimens for the ARIES® *C. difficile* Assay are stable when subjected to up to five freeze thaw cycles.

Shelf-Life Stability

Real time stability was determined for the ARIES® *C. difficile* Assay Cassette in order to establish a shelf life. This was assessed by testing at multiple time points six toxigenic *C. difficile* positive and negative replicates with each of three different lots of ARIES® *C. difficile* Assay Cassettes that had been stored at two different temperatures (4°C and room temperature). All targets for all lots and all storage temperatures gave the expected results. No trend of increased invalid rate was observed over the course of the study and the overall invalid rate was 1.2%. The expected results were observed upon retesting samples with initial Invalid results. Based on this study, a shelf life of 12 months has been assigned to the ARIES® *C. difficile* Assay.

Open Box stability was determined for the ARIES® *C. difficile* Assay Cassettes after they were removed from their individual pouches. Cassettes were removed from their pouches and placed on a laboratory bench where they were exposed to ambient temperatures, humidity, and light for up to 10 hours. Data were collected for toxigenic *C. difficile* Positive and *C. difficile* Negative (ARIES® Stool Resuspension Buffer) samples at five time points. Three lots of cassettes were used to assess open box stability. At the end of 10 hours, all three lots of cassettes produced expected results. An overall invalid rate of 1.6% was observed over the course of the study. The expected results were observed upon retesting samples with initial Invalid results. Therefore the ARIES® *C. difficile* Assay Cassettes are stable in ambient laboratory conditions for up to 10 hours after they have been removed from the storage pouch.

d. Detection Limit:

The limit of detection of the ARIES *C. difficile* Assay was determined by testing dilutions of enumerated stocks of toxigenic *C. difficile* in stool matrix. The Limit of Detection (LoD) was defined as the lowest concentration tested at which ≥95% of assay replicates produced positive results

The confirmed reportable LoD concentrations of each *C. difficile* strain were determined by colony counting and are presented in Table 11.6.

Table 11.6. LoD Results for *C. difficile* strains

			LOD Concentration (CFU) [a]	
***C. difficile* Strain**	**Toxinotype**	***tcdA*/*tcdB* Genes**	**Per mL Stool**	**Per cassette**
ATCC® 43598	**VIII**	-/+	139.9	4.7
ATCC® BAA-1812	**XII**	+/+	110.4	3.7
ATCC® BAA-1803	**IIIc [b]**	+/+	18.6	0.6
ATCC® BAA-1870	**IIIb [b]**	+/+	19.2	0.6
ATCC® BAA-1871	**0**	+/+	31.2	1.0

[a] The number of cfu at LoD concentration was determined by colony counting.
[b] Outbreak-associated Pulsed Field Gel Electrophoresis type NAP1

e. Analytical Reactivity (Inclusivity)

Analytical reactivity performance characteristics for the ARIES® *C. difficile* Assay were assessed by testing 15 strains of toxigenic *C. difficile* in addition to those included in the LoD Study (1 each of toxinotypes V and XXII and 13 toxinotype 0) and an *in-silico* analysis of *C. difficile* toxinotype X. All strains positive for *tcdA* and/or *tcdB* were detected and *in-silico* analysis suggests that strains of toxinotype X will also be detected. In total, analytical testing demonstrated the ability of the ARIES *C. difficile* Assay to detect seven different toxinotypes (0, V, IIIc (Nap1), IIIb, VIII, XII, XXII).

f. Analytical specificity:

Cross-Reactivity and Microbial Interference:

A study was performed to evaluate cross reactivity and interference of the ARIES® *C. difficile* Assay with 61 microorganisms and viruses that might be present in the sample matrix, in addition to human DNA (Table 11.7). Interference was evaluated by testing three replicates each of *C. difficile* strains BAA-1870 and BAA-1871 (both $tcdA^+/tcdB^+$)in the presence of each potentially interfering species. To assess the potential for cross-reaction, toxigenic *C. difficile* Negative replicates (Negative Stool Matrix) were also tested. Testing of potentially cross-reactive or interfering species was performed at a concentration of $\geq 10^6$ cfu/mL of Resuspension Buffer for bacteria and yeast and $\geq 10^5$ $TCID_{50}$/mL for viruses, or the highest available concentration. Human DNA was tested at 5µg/mL of Stool Resuspension Buffer. The results of the study are presented in Table 11.7.

On initial testing, 1/3 replicates for *C. bifermentans* and *E. coli* were reported as positive for toxigenic *C. difficile*. Upon repeat testing of both organisms, 3/3 replicates gave negative results with the ARIES *C. difficile* Assay.

Invalid results were obtained on initial testing of *A. baumannii* (3/3 replicates) and *S. marcescens* (2/3 replicates) at >10^7 CFU/mL. Both produced negative results when retested at 10^6 CFU/mL.

One organism, *C. botulinium,* that was not available for laboratory testing was evaluated by *in-silico* analysis, on the basis of which, it is not expected to cross react or interfere with the ARIES® *C. difficile* Assay.

Table 11.7. Cross Reacting Organisms Tested

Organism Name	Test Concentration (per mL Stool Resuspension Buffer)
Abiotrophia defectiva	5.80 x 10^7 cfu/mL
Acinetobacter baumannii	1.0 x 10^6 cfu/mL
Adenovirus Type 7A	5.12 x 10^6 $TCID_{50}$/mL
Aeromonas hydrophila	7.5 x 10^7 cfu/mL
Alcaligenes faecalis subsp. faecalis	1.1 x 10^9 cfu/mL
Bacillus cereus	4.87 x 10^6 cfu/mL
Bacteroides fragilis	2.39 x 10^8 cfu/mL
Campylobacter coli	2.55 x 10^7 cfu/mL
Campylobacter jejuni	1.44 x 10^6 cfu/mL
Candida albicans	1.33 x 10^7 cfu/mL
Citrobacter freundii	1.45 x 10^8 cfu/mL
Clostridium bifermentans	5.25 x 10^7 cfu/mL
Clostridium butyricum	2.24 x 10^7 cfu/mL
Clostridium haemolyticum [b]	1.29 x 10^5 cfu/mL
Clostridium perfringens	5.30 x 10^6 cfu/mL
Clostridium scindens	8.90 x 10^7 cfu/mL
Clostridium septicum	8.10 x 10^6 cfu/mL
Clostridium sordellii	1.64 x 10^6 cfu/mL
Clostridium sporogenes	5.15 x 10^7 cfu/mL
Clostriduim novyi	1.40 x 10^8 cells/mL
Coxsackievirus (Type A16)	2.04 x 10^6 $TCID_{50}$/mL
Cytomegalovirus (Type AD-169)	5.74 x 10^5 $TCID_{50}$/mL
Echovirus Type 11	2.94 x 10^6 $TCID_{50}$/mL
Edwardsiella tarda	4.42 x 10^7 cfu/mL
Enterobacter aerogenes	8.75 x 10^8 cfu/mL
Enterobacter cloacae	2.99 x 10^8 cfu/mL
Enterococcus faecalis vanB	4.95 x 10^7 cfu/mL
Enterovirus (Type 71) [b]	2.08 x 10^4 $TCID_{50}$/mL
Escherichia coli (026:H4)	1.80 x 10^8 cfu /mL

Organism Name	Test Concentration (per mL Stool Resuspension Buffer)
Escherichia coli (O157:H7)	2.05×10^8 cfu /mL
Flavonifactor plautii [a]	3.07×10^7 cfu/mL
Helicobacter pylori	9.80×10^6 cfu/mL
Human genomic DNA	5 µg/mL
Klebsielia oxytoca	5.20×10^8 cfu/mL
Lactobacillus acidophilus	1.25×10^7 cfu/mL
Listeria monocytogenes	4.65×10^8 cfu/mL
Non-toxigenic *Clostridium difficile* strain 43593	5.05×10^6 cfu/mL
Non-toxigenic *Clostridium difficile* strain 43601	1.01×10^7 cfu/mL
Non-toxigenic *Clostridium difficile* strain 43602	3.17×10^6 cfu/mL
Non-toxigenic *Clostridium difficile* strain 43603	4.05×10^6 cfu/mL
Norovirus Group I	4.26×10^6 $TCID_{50}$/mL
Norovirus Group II	4.26×10^6 $TCID_{50}$/mL
Peptostreptococcus anaerobius	2.29×10^6 cfu/mL
Plesiomonas shigelloides	1.52×10^8 cfu/mL
Porphyromonas asaccharolytica	3.70×10^6 cfu/mL
Prevotella melaninogenica	2.05×10^6 cfu/mL
Proteus mirabilis	1.42×10^8 cfu/mL
Providencia alcalifaciens	2.07×10^8 cfu/mL
Pseudomonas aeruginosa	1.97×10^8 cfu/mL
Rotavirus[b]	8.49×10^3 $TCID_{50}$/mL
Salmonella enterica (typhimurium)	5.95×10^8 cfu/mL
Salmonella enterica subsp. arizonae	5.80×10^8 cfu/mL
Salmonella enterica subsp. enterica	2.60×10^8 cfu/mL
Serratia liquefaciens	5.45×10^8 cfu/mL
Serratia marcescens	1.00×10^6 cfu/mL
Shigella boydii	2.32×10^8 cfu/mL
Shigella dysentariae	1.59×10^8 cfu/mL
Shigella sonnei	1.15×10^8 cfu/mL
Staphylococcus aureus	5.45×10^8 cfu/mL
Staphylococcus epidermidis	1.45×10^8 cfu/mL
Streptococcus agalactiae	8.25×10^7 cfu/mL
Vibrio parahaemolyticus	1.07×10^8 cfu/mL

[a] Same species as *Clostridium orbisceindens*.
[b] Tested at the highest available concentration provided by Zeptometrix Corporation.

Interfering Substances:

The effect of potential interfering substances on the ARIES® *C. difficile* Assay was evaluated by spiking prepared solutions of potential interfering substances, presented in Table 11.8, into stool matrix with and without toxigenic *C. difficile*. For each potentially interfering substance, three replicates of each of two strains of toxigenic *C. difficile* (both *tcdA*$^+$/*tcdB*$^+$) were tested, in addition to three *C.*

difficile negative samples. A total of 14 interfering substances were tested for inhibitory effects on the ARIES® *C. difficile* Assay. False negative results were obtained in the presence of mucin at 3.5% w/v, although no interference was observed when mucin was tested at 0.35% w/v. In the presence of all other substances tested, *C. difficile* was detected in 100% of replicates.

Table 11.8. Interfering Substances Tested

Interfering Substance	Concentration of Interfering Substance in Stool
Barium sulfate	1.3% w/v
Fecal fat (Triglyceride)	20.0% w/v [a]
Fecal fat (Cholesterol)	4.9% w/v
Hemoglobin (tarry stool)	12.5% w/v
Hydrocortisone Cream	2.0% w/v
Imodium	0.63% w/v [b]
Kaopectate	0.1% w/v
Metronidazole	140.0 mg/mL [a]
Moist towelettes (Benzalkonium Chloride)	10.0% v/v
Mucin	0.35% w/v [c]
Pepto-Bismol	0.1% w/v [a]
Preparation H	2.0% w/v [d]
Vagisil anti-itch cream	2.0% w/v
Whole Blood	20% v/v

[a] 1/3 replicates for BAA-1871 was negative for *tcdA*
[b] 2/3 replicates for BAA-1870 were negative for *tcdA*
[c] False negative results may be observed in the presence of mucin at concentrations >0.35%
[d] 2/3 replicates for both BAA-1870 and BAA-1871 were negative for *tcdA*

Carry-Over/Cross-Contamination:

Carry-over and cross contamination for the ARIES® *C. difficile* Assay was assessed by testing 30 high positive *C. difficile* samples and 30 *C. difficile* negative samples (Negative Stool Matrix). Samples were tested in an alternating pattern with high positive samples run adjacent to negative samples across 11 consecutive runs. No carry-over or cross contamination was observed. The overall percent agreement was 100% for positive and negative samples.

g. Assay cut-off

For the ARIES® *C. difficile* Assay, each target (*tcdA* and *tcdB*) has a Ct cut-off, Tm window, and Tm Peak Threshold. In addition, the internal sample processing control (SPC) also has a corresponding Ct cut-off, Tm window, and Tm Peak Threshold. Collectively, the cut-off values compose the assay protocol file parameters, which are used to determine the assay result for the detection target as POSITIVE, NEGATIVE, or INVALID. These values are hard-coded into the

ARIES® *C. difficile* Assay protocol file and are not modifiable. The Assay Protocol File parameters were determined, and their performance in the ARIES® *C. difficile* Assay evaluated according to the following general procedure:

- Initial Assay Protocol File parameters were set during internal optimization studies
- The final Assay Protocol File parameters were then established during internal verification studies
- The selected Assay Protocol File parameter values were utilized in the determination of assay performance in the multi-site clinical trial conducted for the ARIES C. difficile Assay

The specific assay parameters for the ARIES® *C. difficile* Assay are considered **confidential and proprietary**.

2. Clinical Performance:

Performance of the ARIES® *C. difficile* Assay was evaluated prospectively from 31-October-2016 to 21-February-2017 at four geographically distinct clinical sites within the United States using the ARIES® System. Specimens for the clinical study consisted of excess leftover de-identified, unpreserved, unformed stool specimens from patients suspected of having *Clostridium difficile* infection (CDI). All eligible leftover stool specimens were tested with a reference method (direct and enriched toxigenic culture) and ARIES® *C. difficile* Assay and the results compared. Reference method testing was performed at a centralized testing facility while ARIES® *C. difficile* Assay testing was performed at each clinical site on their own clinical specimens.

A total of 1021 stool specimens from subjects suspected of having CDI were collected from four geographically diverse locations within the United States. Of these 1021 specimens, 37 were excluded from the study based on inclusion/exclusion criteria leaving a total of 984 unique specimens that met the predetermined inclusion criteria and that were included in the data analysis. These 984 specimens were enrolled in the study and tested for toxigenic *C. difficile* by both the reference method of direct and enriched toxigenic culture and the ARIES® *C. difficile* Assay. There were 28 specimens (28/984, 2.8%) that were re-tested with ARIES® *C. difficile* Assay because they yielded initial invalid results due to run failure or instrument error. An additional 15 specimens were re-tested with the ARIES® *C. difficile* Assay because of either sample mix-up (N=5) or improper sample storage or processing (N=10). Thirty-eight (38) of the 43 specimens that were re-run generated valid ARIES® *C. difficile* Assay results (i.e. positive or negative) after re-test. Five (5)

specimens remained invalid by ARIES® *C. difficile* Assay upon re-test for an overall invalid rate after repeat testing of 0.5% (5/984).

For the 979 eligible specimens that were included in the device performance calculations, positive percent agreement of the ARIES® *C. difficile* Assay for toxigenic *C. difficile* against direct toxigenic culture was 98.1% (103/105) with a lower bound 95% confidence interval of 93.3%. When compared to direct and enriched toxigenic culture, clinical sensitivity of the ARIES® *C. difficile* Assay for toxigenic *C. difficile* was 90.5% (133/147) with a lower bound 95% confidence interval of 84.6%. Negative percent agreement of the ARIES® *C. difficile* Assay for toxigenic *C. difficile* in comparison to direct toxigenic culture was 92.6% (809/874, Lower Bound 95% CI, 90.6%), while clinical specificity in comparison to direct and enriched culture was 95.8% (797/832, Lower Bound 95% CI, 94.2%).

Table 11.9. ARIES® *C. difficile* Assay Performance Compared to Direct Culture (N=979)

ARIES® *C. difficile* Assay	**Direct Toxigenic Culture**		
	Positive	Negative	TOTAL
Positive	103	65[2]	168
Negative	2[1]	809	811
TOTAL	105	874	979[3]
		95% CI	
Positive Percent Agreement	98.1%	93.3% - 99.5%	
Negative Percent Agreement	92.6%	90.6% - 94.1%	

[1] One of the ARIES® *C. difficile* Assay negative specimens that was positive by direct toxigenic culture (i.e. False Negative) was *C. difficile* negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® *C. difficile* Assay.

[2] Of the 65 ARIES® *C. difficile* Assay positive specimens that were negative by direct toxigenic culture (i.e. False Positive), 30 were positive by enriched toxigenic culture. An additional 15 specimens were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® *C. difficile* Assay.

[3] Five (5) specimens generated invalid results by the ARIES® *C. difficile* Assay after allowable re-run. Four (4) of these were negative and one (1) was positive by direct toxigenic culture. All of these 5 specimens were excluded from the device performance calculations.

Table 11.10. ARIES® *C. difficile* Assay Performance Compared to Direct and Enriched Toxigenic Culture (N=979)

ARIES® *C. difficile* Assay	Direct and Enriched Toxigenic Culture		
	Positive	Negative	TOTAL
Positive	133	35[2]	168
Negative	14[1]	797	811
TOTAL	147	832	979[3]
		95% CI	
Sensitivity	90.5%	84.6% - 94.2%	
Specificity	95.8%	94.2% - 97.0%	

[1] Thirteen (13) of the ARIES® *C. difficile* Assay negative specimens that were positive by direct and enriched toxigenic culture (i.e. False Negative) were *C. difficile* negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® *C. difficile* Assay

[2] Fifteen (15) of the ARIES® *C. difficile* Assay positive specimens that were negative by enriched toxigenic culture (i.e. False Positive) were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® *C. difficile* Assay.

[3] Five (5) specimens generated invalid results by the ARIES® *C. difficile* Assay after allowable re-run. Four (4) of these were negative and one (1) was positive by direct and enriched toxigenic culture. All of these 5 specimens were excluded from the device performance calculations.

The study results demonstrate that the diagnostic accuracy of the ARIES® *C. difficile* Assay is acceptable for the detection of *C. difficile* in unpreserved, soft or liquid stool specimens from patients suspected of having *Clostridium difficile* infection (CDI).

3. Expected values/Reference range:

The prevalence of toxigenic *C. difficile* observed during a multi-center clinical trial using the ARIES *C. difficile* Assay was estimated as 17.2% (168/979). Of the patient populations included in the study, the majority of patients were senior adults (≥60 years) and the prevalence of *C. difficile* in this age group was found to be 18.1% (88/487). The second largest age group was adults (age 22 to <60 years) and the prevalence was found to be 16.1% (74/460). The next age group was adolescents (12 to <22 years) and the prevalence was found to be 22.2% (6/27). The remaining age group included 4 children (2 to <12 years) and one infant (<2 years) where the prevalence was found to be 0%.

N. Proposed Labeling:

The labeling provided in the submission satisfies the requirements of 21 CFR 809.10.

O. Conclusion:

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