Luminex

Package Insert | IVD

ARIES® *C. difficile* Assay

IVD For *In Vitro* Diagnostic Use.

For Use With ARIES® Systems.

Rx Only

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ARIES® *C. difficile* Assay Package Insert

89-30000-00-484 Rev. A
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CE

EC REP

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Key to Symbols

5.1.4* 	Use-by date Indicates the date after which the medical device is not to be used.	5.3.7* 	Temperature Limit Indicates the temperature limits to which the medical device can be safely exposed.
5.1.5* 	Batch Code Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.4.2* 	Do not reuse Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure.
5.1.6* 	Catalog(ue) Number Indicates the manufacturer's catalogue number so that the medical device can be identified.	5.4.4* 	Caution Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
5.1.1* 	Manufacturer Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.	5.5.5* 	Contains Sufficient for <n> Tests Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
5.4.3* 	Consult instructions for use. Indicates the need for the user to consult the instructions for use.	5.4.1* 	Biological Hazard
BC	Build Code	GHS02† 	Highly flammable liquid and vapor
5.2.8* 	Do not use if package is damaged. Indicates a medical device that should not be used if the package has been damaged or opened.	5.5.1* 	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

5.1.2* EC REP	Authorized representative in the European Community Indicates the Authorized representative in the European Community.	‡ CE	Conformite Europeenne (EU CE Marking of Conformity) Council Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDMD) (1998)
§ Rx Only	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only). 21 CFR 809 (FDA Code of Federal Regulations)	0434B ‖ 	Caution To indicate that caution is necessary when operating the device or control close to where the symbol is placed, or to indicate that the current situation needs operator awareness or operator action in order to avoid undesirable consequences.
5.1.7* SN	Serial Number indicates the manufacturer's serial number so that a specific medical device can be identified.		

* ANSI/AAMI/ISO 15223-1:2012, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† ST/SG/AC.10/30/Rev.6 Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Sixth revised edition.

‡ Council Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDMD) (1998).

§ 21 CFR 809 (FDA Code of Federal Regulations).

‖ ISO 7000: Fifth edition 2014-01-15, graphical symbols for use on equipment - registered symbols. (General I (QS/RM)).

Table of Contents

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.

Summary and Explanation of the Test

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming bacterium usually spread by the fecal-oral route. It is non-invasive and produces toxins A and B that cause disease, ranging from asymptomatic carriage, to mild diarrhea, to colitis, or pseudomembranous colitis (Surawicz *et al*., 2013). *Clostridium difficile* infection (CDI) is a leading cause of hospital associated gastrointestinal illness and places a high burden on our health-care system, with costs of 3.2 billion dollars annually (McFarland 2011 and O'Brien, Lahue *et al*., 2007).

The major virulence factors for *C. difficile* are toxin A and toxin B that are encoded by the *tcdA* and *tcdB* gene respectively. Most pathogenic strains of *C. difficile* are either A+B+ or A-B+ (Sambol, Merrigan *et al*. 2000). Recently, there have been reports of new strains that only produce *tcdA* (A+B-) (Monot *et al*., 2015). Unlike many other molecular assays, the ARIES® *C. difficile* Assay is able to detect both *tcdA* and *tcdB*. Older adults who take antibiotics and get medical care have the highest risk of *C. difficile* infection (CDC 2015). The tissue culture cytotoxic assay is considered the gold standard for *C. difficile* testing but the procedure is slow and labor intensive (Keller and Weber 2014). Toxin A+B EIA's have also been widely used to test for the presence of toxin but these tests have reduced sensitivity compared to the gold standard (Surawicz, *et al*., 2013). Recent advancements in PCR technology have allowed the development of stand-alone tests based on nucleic acid amplification that offer high sensitivity and specificity (Sloan, Duresko *et al*. 2008).

The ARIES® *C. difficile* Assay uses Luminex Corporation's real-time PCR chemistry in combination with ARIES® Systems. ARIES® Systems are capable of automated nucleic acid extraction and purification, real-time PCR detection of nucleic acid sequences, and data analysis.

Principles of the Procedure

Unpreserved, raw stool sample is processed using the ARIES® Stool Resuspension Kit. ARIES® Stool Resuspension Buffer is added to the ARIES® Stool Resuspension Tube. Primary stool sample is then added to the ARIES® Stool Resuspension Tube containing the ARIES® Stool Resuspension Buffer. The sample is vortexed, centrifuged, and then added to the sample chamber of an ARIES® *C. difficile* Assay cassette. The cassette is then placed into an ARIES® magazine which can hold up to six cassettes. The magazine is inserted into an ARIES® instrument. A barcode on top of the ARIES® *C. difficile* Assay cassette is automatically scanned by the ARIES® instrument, associating a preloaded ARIES® *C. difficile* Assay protocol file with the cassette. The ARIES® *C. difficile* Assay protocol file contains the necessary parameters to run the cassette, analyze data, and generate reports.

Once a run is started, the sample processing control (SPC) is automatically added to the sample chamber of the cassette to control for recovery of extracted nucleic acid, detection of inhibitory substances, and confirmation of PCR reagent integrity. Sample and SPC lysis, as well as isolation and purification of nucleic acids, are automated within ARIES® Systems and the ARIES® *C. difficile* Assay cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized *C. difficile* Master Mix for the PCR amplification step. The *C. difficile* Master Mix contains primer pairs specific to *tcdA*,

tcdB, and the SPC sequence. Total assay time, including extraction and PCR cycling, takes approximately 2 hours.

Materials Provided

The ARIES® *C. difficile* Assay Complete Kit (Part Number 50-10023) includes 24 assay cassettes and the ARIES® Stool Resuspension Kit**.**

The assay protocol file, package insert, and *ARIES® Quick Guide* ship separately on a USB as part of the ARIES® *C. difficile* Assay Protocol File Kit (CN-0334-01).

TABLE 1. **ARIES® *C. difficile* Assay Contents**

Item	Number	Description
ARIES® *C. difficile* Assay Complete Kit	50-10023	24 ARIES® *C. difficile* Assay cassettes which contain necessary reagents for sample extraction, nucleic acid purification, amplification, and the ARIES® Stool Resuspension Kit.
ARIES® *C. difficile* Assay Protocol File Kit	CN-0334-01	An assay protocol file, a package insert, and an *ARIES® Quick Guide* containing instructions for use, are provided on a USB.

The following ancillaries are required and provided with the ARIES® *C. difficile* Assay Complete Kit (50-10023). Additional quantities may be ordered, if desired, using the part numbers below:

- ARIES® Stool Resuspension Kit (PN:30-00095)
- ARIES® Stool Resuspension Buffer (PN:30-00103)

Materials Required But Not Provided

Equipment:

- Appropriately sized pipettor for dispensing 200 µL to 800 µL volumes
- Vortex mixer
- Microcentrifuge
- Luminex® ARIES® Systems (either an ARIES® System or an ARIES® M1 System can be used) and accessories
  - ARIES® magazines
  - Sample Prep Tray
  - Hand-held barcode reader

Plasticware and Consumables

- Nuclease-free aerosol-barrier pipette tips

Warnings and Precautions

1. For *In Vitro* Diagnostic Use.
2. For prescription use only.
3. Handle all samples as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*, and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*.
4. Wash hands thoroughly after performing the test.

5. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
6. Thoroughly clean and disinfect all surfaces with 10% bleach.
7. Avoid contamination from positive controls and samples by following good laboratory practices.
8. Avoid contamination by using a new nuclease-free aerosol barrier tip and swab to add ARIES® Stool Resuspension Buffer and primary sample to the device.
9. Discard the ARIES® Stool Resuspension Tube after use. If a re-test is needed, process the sample using a new ARIES® Stool Resuspension Tube.
10. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash hands thoroughly after performing the test.
11. Follow your institution's safety procedures for working with chemicals and handling biological samples.
12. Dispose of unused reagents and waste in accordance with county, federal, provincial, state and local regulations.
13. Do not use cassettes, kits, or reagents beyond their expiration date.
14. The cassettes are single-use. Do not reuse cassettes.
15. Do not use kit components that appear to be broken or damaged.
16. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
17. Only use the assay protocol file provided by Luminex on the USB drive.
18. Only use the procedures described in this package insert. Any deviation from the outlined procedures can result in assay failure or cause erroneous results.
19. Only prepare specimens for testing using the ARIES® Stool Resuspension Swab. Do not use alternative types of swab. Carefully follow the instructions for Sample Processing to ensure transfer of an appropriate quantity of stool to the Stool Resuspension Tube.
20. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
21. ARIES® cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheet (SDS) regarding safe handling practices for any spills.
22. In the event that a PCR tube falls off the cassette or a cassette leaks inside the ARIES® instrument, you should perform appropriate decontamination procedures to reduce the risk of contamination.

    Immediately clean all surfaces of the ARIES® magazine and the surrounding bench top with water. Wipe the surfaces with a lint-free cloth. Follow that with a fresh 10% bleach solution. Allow the bleach solution to sit for a minimum of 10 minutes. Thoroughly rinse bleached surfaces with deionized water. Dispose of all lint-free cloths in the appropriate waste container. Immediately contact Luminex Technical Support in order to retrieve the PCR tube from the ARIES® instrument. Do not throw away the cassette before you contact Technical Support. Do not attempt to retrieve the tube or put your hands inside the ARIES® instrument at any time. Do not proceed with additional testing until the PCR tube has been removed from the ARIES® instrument. Discard the cassette in accordance with the procedures defined by appropriate biohazard safety guidelines or regulations.
23. Refer to the appropriate ARIES® system operation manual for electrical and mechanical warnings.
24. Do not let the ARIES® Systems get wet or allow standing water to pool under the instrument.
25. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Reagent Storage, Handling, and Stability

NOTE: If the ARIES® Stool Resuspension Buffer is frozen on receipt, thaw to room temperature, invert the bottle to mix, and continue testing.

ARIES® *C. difficile* Assay cassettes are shipped refrigerated. Store at room temperature (15°C to 30°C) after receipt. The ARIES® Stool Resuspension Buffer ships at an ambient temperature and is stored at room temperature.

Always check the expiration date on the kit boxes and cassettes.

Sample Handling and Storage

Sample Collection

Fresh stool samples should be placed in sterile, leak-proof, wide-mouthed, preservative-free containers. Follow your institution's guidelines for collecting *C. difficile* samples for testing.

Sample Transport

When transporting biological samples, ensure that all applicable regulations for the transport of etiologic agents are met.

Unpreserved raw stool samples should be transported to the laboratory in a refrigerated state (2°C to 8°C).

Sample Storage

Samples can be stored at 15°C to 30°C (room temperature) for up to 4 hours or 2°C to 8°C (refrigerated) for up to 7 days. If the sample cannot be tested within the indicated durations at the specified temperatures, then the sample can be stored at ≤ -70°C for up to three months.

Software Setup

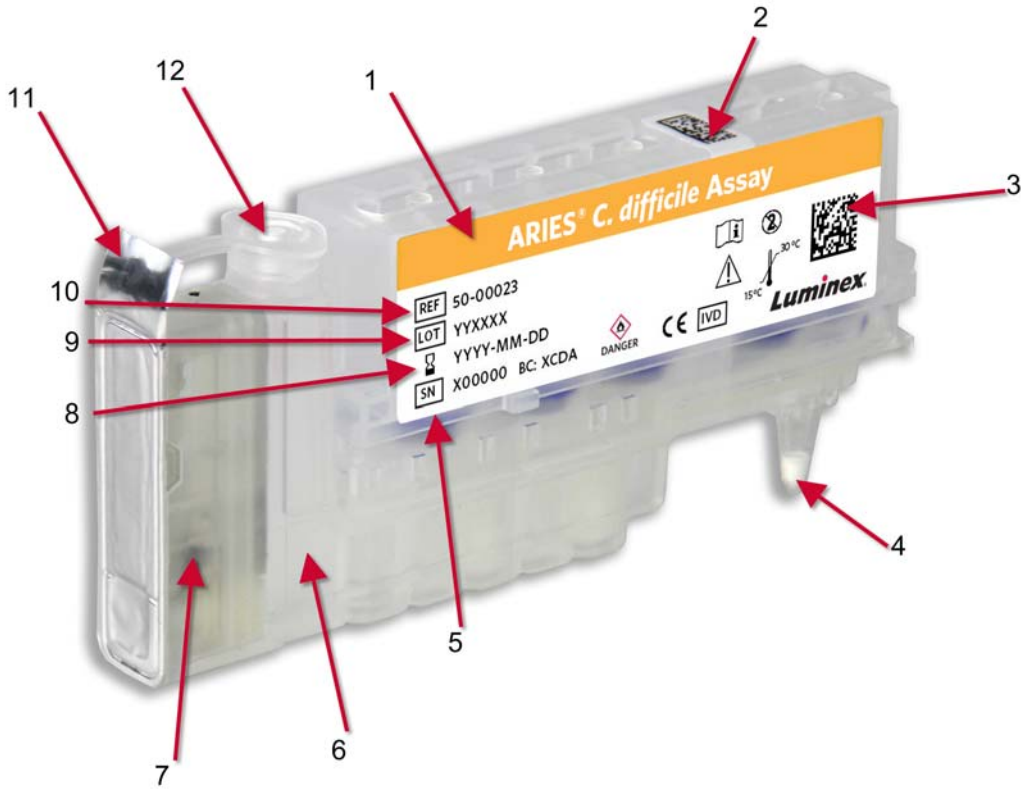
Importing Assay Files to ARIES® Systems

The ARIES® *C. difficile* Assay protocol file is provided on the USB flash drive. The assay protocol file only needs to be imported to ARIES® Systems once. To import the assay protocol file, complete the following:

1. Insert the USB flash drive into one of the five USB connectors (one in the front and four in the back).
2. Select  in the upper left-hand corner of the screen and navigate to **Assay Management**.
3. Select 

from the Page Action bar. The **Import File** dialog box displays.
4. Choose the **Location** and **File Name** of the assay file. Select **OK**.

FIGURE 1. **ARIES® Cassette**



1. Assay type	7. Side cassette
2. Cassette barcode (top)	8. Cassette expiration date
3. Cassette barcode (side)	9. Cassette lot number
4. PCR tube	10. Cassette part number
5. Cassette serial number	11. Back seal
6. Cassette sample chamber	12. Cassette cap

Assay Procedure

Sample Processing

1. Label the ARIES® Stool Resuspension Tube with the Sample ID.
2. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 800 µL of ARIES® Stool Resuspension Buffer to the ARIES® Stool Resuspension Tube.
3. Observe the consistency of the stool specimen. Collect the appropriate stool specimen using the provided ARIES® Stool Resuspension Swab, according to the specimen's consistency:
   - Soft Stool Specimen (Bristol Score 5-6)
     a. Insert the ARIES® Stool Resuspension Swab into the stool specimen and roll the swab over the specimen to fully coat.
     b. Once the ARIES® Stool Resuspension Swab is fully coated, roll the swab on the inside wall of the specimen collection container to remove excess specimen.

NOTE: There should be a thin coating around the entire ARIES® Stool Resuspension Swab.

Appropriate amount of soft specimen:

Inappropriate amount of soft specimen:

- Liquid Stool Specimen (Bristol Score 7):
  a. Vortex the stool specimen for 5 seconds until well mixed.
  b. Swirl the ARIES® Stool Resuspension Swab in the stool specimen for approximately 5 seconds to saturate the swab.
  c. Tap the ARIES® Stool Resuspension Swab on the inside wall of the specimen collection container to remove excess specimen.

NOTE: The ARIES® Stool Resuspension Swab should not drip liquid.

Appropriate amount of liquid specimen:

Inappropriate amount of liquid specimen:

4. Place the ARIES® Stool Resuspension Swab into the ARIES® Stool Resuspension Tube that contains 800 µL of ARIES® Stool Resuspension Buffer.
   a. Line up the breakpoint of the swab shaft with the rim of the tube and break the ARIES® Stool Resuspension Swab from the shaft by applying firm but gentle force to the swab shaft against the ARIES® Stool Resuspension Tube rim.

   NOTE: Gripping the ARIES® Stool Resuspension Swab shaft closer to the breakpoint and rotating while bending, makes breaking the shaft off easier. Gently twirl the ARIES® Stool Resuspension Swab if the shaft and swab remain slightly attached after breaking.

b. With the ARIES® Stool Resuspension Swab remaining in the ARIES® Stool Resuspension Tube, recap the tube.

CAUTION: If gloves become contaminated with stool specimen during transfer to the ARIES® Stool Resuspension Tube, change gloves to avoid specimen contamination.

5. Vortex the ARIES® Stool Resuspension Tube at the highest setting for 15 seconds. Ensure full vortex is formed during mixing.
6. Centrifuge the ARIES® Stool Resuspension Tube at a minimum of 2,000 x g for a minimum of 30 seconds.
7. Repeat Steps 1-6 for remaining specimens to be tested.

Adding Samples to Cassettes

1. Remove the assay cassette from its packaging and visually inspect the cassette for any damage.

CAUTION: If the cassette(s) or its packaging appears damaged in any way or if you see any leaks, DO NOT USE THE CASSETTE. Immediately contact Luminex Technical Support to report the damage.

2. Close the cassette cap to seal the cassette sample chamber.
3. Place the ARIES® Stool Resuspension Tube in the Sample Prep Tray.
4. Pull the tab to remove the foil seal from the cassette.

CAUTION: Use caution when pulling the back seal off the cassettes. The foil is sharp and may cause injury.

5. Place the cassette in the Sample Prep Tray next to the sample.
6. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 200 µL of processed stool sample from the ARIES® Stool Resuspension Tube. Pipette from the top of the processed stool sample to avoid disturbing any beads that settle to the bottom of the ARIES® Stool Resuspension Tube.

CAUTION: Verify that the pipette is correctly set to aspirate 200 µL. Adding a higher volume of processed stool sample to the cassette may result in assay inhibition.

CAUTION: Avoid disturbing the beads contained in the ARIES® Stool Resuspension Tube or transferring the beads to the cassette. Use care to avoid contamination of the pipettor during transfer of the processed stool sample from the ARIES® Stool Resuspension Tube to the cassette.

CAUTION: If the sediment layer is disturbed, recap and centrifuge the ARIES® Stool Resuspension Tube at a minimum of 2,000 x g for a minimum of 30 seconds and pipette 200 µL of processed stool sample.

7. Open the cassette cap and place the processed stool sample in the cassette sample chamber by inserting the pipette tip near the bottom of the chamber before expelling the processed stool sample.
8. Close the cassette cap to seal the cassette sample chamber.

   WARNING: Failure to ensure the cassette cap is fully closed may cause a delay or failure in results and expose you to biohazards.

   CAUTION: Do not vortex or shake the cassette.
9. Repeat steps 1-8 for remaining specimens to be tested.
10. Immediately proceed to order entry and initiation of the run on the ARIES® System.

    WARNING: If run initiation is delayed, prepare a new sample and test cassette beginning from Sample Processing Step 1, above.

Entering Orders on ARIES® Systems

When entering orders, the Sample ID and Assay are required for an order to be valid.

NOTE: The order should be created prior to placing the cassette in the magazine. If you scan the cassette while the cassette is in the magazine, it is possible to scan the incorrect cassette barcode.

1. Select  in the upper left-hand corner of the screen and navigate to **Order Management > Sample Orders**.
2. Select 

from the Page Action bar. The **New Order** dialog box displays.
3. Pick up and scan the barcode on the top (or side) of the cassette with the hand-held barcode reader or enter the required cassette information manually. A touch screen keyboard or a drop-down menu displays.

   NOTE: If the keyboard does not automatically appear, click the toggle next to the keyboard icon to **Yes**. The keyboard will appear when you click in a field.

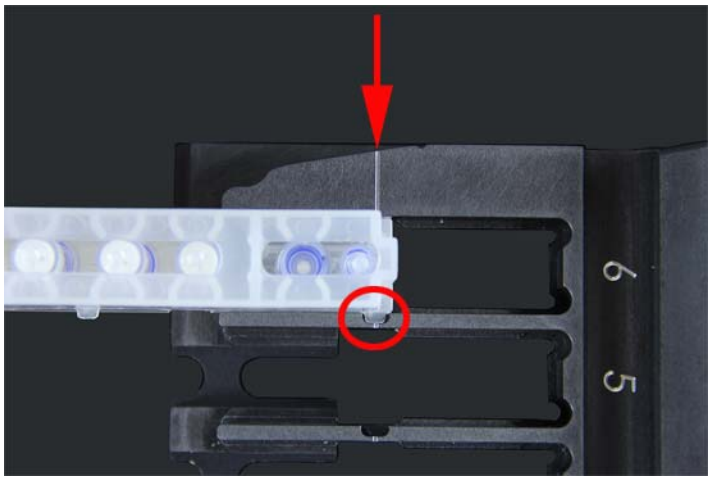
   NOTE: If manually entering the **Cassette Lot Expiration**, select the calendar icon and choose the date using the calendar. The date is shown in the YYMMDD format.

   a. If applicable, to add a control, choose **Control** in the **Sample Type** drop-down menu.
   b. In the **Control** field, click the magnifying glass to select a control from the **Controls** dialog box.
   c. Select the type of control in the **Control Type** drop-down menu.

   NOTE: You can define the controls on the **Assay Management > Controls** page.
4. From the ARIES® Stool Resuspension Tube, pick up and scan the Sample ID with the hand-held barcode reader or enter the required information manually.

   NOTE: Ensure that the ARIES® cassette is associated with the corresponding sample by manually verifying the information on the screen.
5. Scan the **Data Matrix** barcode on the screen next to **Save**, or manually select **Save**.

6. Place the cassette into the magazine by lining the cassette up with the first notch (a tab on the cassette fits into the notch).



NOTE: The PCR tube must face toward the numbers on the magazine.

7. Gently insert the cassette into the magazine.
8. Gently slide the cassette all the way back toward the numbers. Repeat for all other cassettes.

WARNING: Do not use your index finger to push the cassette into the magazine. You may indirectly dispense the reagent. Luminex recommends using the palm of your hand, or holding the cassette and sliding the cassette into proper position.

Running an Assay

1. Select ☰ in the upper left-hand corner of the screen and navigate to **Run >Run**.
2. Insert the magazine into the ARIES® instrument. The ARIES® instrument automatically scans the barcode printed on the top of the ARIES® *C. difficile* Assay cassettes, identifies associated orders, and the proper assay protocol files before starting the run.

   NOTE: Ensure that the **Auto run upon Magazine Insertion** is toggled to **Yes** in the **Run Options** dialog box, located on the **Run Settings** page. The instrument automatically scans the cassettes once the magazine is inserted and starts the run.

3. If there are any errors, the ARIES® instrument displays the specific error (for example, cassettes that cannot be run together, cassette IDs that have not been read, or assay files not loaded on to the ARIES® instrument). These errors must be corrected in order for the run to begin.

a. If **Auto run upon Magazine Insertion** is enabled and no errors occur, the instrument will automatically scan and start the run for you. The magazine state then indicates **PLEASE DO NOT REMOVE THE MAGAZINE** and an orange lock icon displays on the left-hand side of the magazine state. The Run Status bar, located at the bottom of the **Run** page, displays an orange progress bar next to the estimated time to completion, colored purple. If you do not have the Auto Run feature enabled, immediately start the run manually by selecting 

from the Page Action bar.

NOTE: If you are using an ARIES® System with two modules, highlight the module you want before selecting **Start Run**.

Monitoring the Run

From the Run page, select on the Page Action bar to display the status of the magazine(s), the estimated time to completion, and the customizable name of the ARIES® instrument. This status screen is intended to be visible from across the room, allowing you to monitor your runs while you are working on other projects.

TIP: On the Run > Settings page, you can customize whether the estimated completion time or estimated time remaining displays.

Reports and Results

Refer to the appropriate ARIES® system operation manual regarding reports and results.

Interpretation of Sample Results

The ARIES® software determines results for the sample and the sample processing control (SPC) for each sample based on the amplification cycle (Ct) value and the melt parameters (Tm and Peak Fluorescence) provided in the assay protocol file. All assay outcomes are described below. *C. difficile* positivity is based on detection of the *tcdA* and/or *tcdB* target. Results for *tcdA* and *tcdB* are not reported separately.

TABLE 2. **Interpretation of Sample Results**

Example	SPC		*tcdA*		*tcdB*		Toxigenic *C. difficile* Call
	Ct	Melt	Ct	Melt	Ct	Melt	
1	N/A	+	+	+	+	+	Toxigenic *C. difficile* Positive
2	N/A	+	N/A	N/A	+	+	Toxigenic *C. difficile* Positive
3	N/A	+	+	+	N/A	N/A	Toxigenic *C. difficile* Positive
4	+	+	>	N/A	>	N/A	Toxigenic *C. difficile* Negative
5	+	+	-	-	>	N/A	Toxigenic *C. difficile* Negative
6	+	+	>	N/A	-	-	Toxigenic *C. difficile* Negative
7	+	+	-	-	-	-	Toxigenic *C. difficile* Negative
8	-	+	>	N/A	>	N/A	Invalid
9	-	+	-	N/A	>	N/A	Invalid
10	-	+	>	N/A	-	N/A	Invalid
11	-	+	-	N/A	-	N/A	Invalid
12	N/A	+	-	-	-	+	Invalid
13	N/A	+	-	+	-	-	Invalid
14	N/A	+	-	+	-	+	Invalid
15	N/A	-	N/A	N/A	N/A	N/A	Invalid

Legend	
+	Value meets acceptance criteria
-	Value does not meet acceptance criteria
N/A	Not applicable. All outcomes will result in the same call.
>	Indicates that the Ct is beyond the Ct cutoff

Invalid Results

In case of an “Invalid” result, re-test beginning with the primary sample. Start at *“Assay Procedure”* on page 5 and use a new assay cassette and ARIES® Stool Resuspension Tube. If the problem is unresolved, contact Luminex Technical Support.

Quality Control

Quality control procedures intended to monitor the ARIES® Systems and assay performance are outlined in *Table 3*.

TABLE 3. **Controls to Monitor Quality**

Control Type	Use
Sample Processing Control	Verifies nucleic acid extraction, and proper reagent, cassette, ARIES® instrument, and assay protocol performance.

Each ARIES® *C. difficile* Assay cassette contains a sample processing control, which is processed with the sample and analyzed during the amplification reaction.

External controls may be used in accordance with local, state, federal accrediting organizations, as applicable. A toxigenic reference strain of *C. difficile* or clinical isolate may be used as a Positive Control. Another species of *Clostridium* or 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.

Limitations

1. The detection of bacterial nucleic acids depends on proper sample collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to an incorrect result.
2. There is a risk of false negative results due to improperly collected, transported, or handled raw stool samples.
3. The ARIES® *C. difficile* Assay is for use with unpreserved, liquid or soft human stool specimens from symptomatic patients. Performance characteristics with other specimen types or patients without symptoms of infection with toxigenic *C. difficile* have not been established.
4. A negative ARIES® *C. difficile* Assay result does not rule out the possibility of infection with toxigenic *C. difficile*. ARIES® *C. difficile* Assay results should not be used as the sole basis for diagnosis, treatment, or patient management decisions and should be interpreted in conjunction with other clinical and laboratory findings.
5. The performance of the ARIES® *C. difficile* Assay has not been established for monitoring treatment of *C. difficile* infection.
6. The ARIES® *C. difficile* Assay detects but does not differentiate hypervirulent NAP1 strains from other toxigenic *C. difficile* genotypes.
7. False negative results may occur due to the presence of sequence variation in the regions of the *tcdA* and *tcdB* genes targeted by the ARIES® *C. difficile* Assay, procedural errors, amplification inhibitors in samples, or the presence of an inadequate quantity of target DNA for amplification.
8. Based on analytical studies, the ARIES® *C. difficile* Assay may exhibit reduced sensitivity for detection of *tcdA*$^+$/*tcdB*$^-$ strains which, although rare, have been associated with CDI (Monot *et al*., 2015).
9. There is a risk of false positive results due to potential cross-contamination by target organism(s), their nucleic acid or amplified product, or from non-specific signals in the assay. Cross-reactivity with or interference by organisms other than those tested could lead to erroneous results.
10. In analytical studies, false negative and Invalid and results were obtained in the presence of very high concentrations (~10^8 CFU/mL) of *Serratia marcescens*. Invalid results were also obtained in the presence of very high concentrations (>10^7 CFU/mL) of *Acinetobacter baumannii.*
11. The potential for assay interference has only been evaluated with *tcdA*$^+$/*tcdB*$^+$ strains of toxigenic *C. difficile* in the presence of the substances listed within the labeling and at the concentrations shown.

These studies demonstrated that false negative results may occur in the presence of mucin at concentrations greater than 0.35% w/v. Interference by substances other than those tested or at higher concentrations could lead to erroneous results.

12. This test has been evaluated for use with human specimen material only.
13. This test is a qualitative test and does not provide quantitative values of the detected organism.
14. This test cannot rule out diseases caused by other bacterial pathogens.
15. For use only on the ARIES® System or ARIES® M1 System.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Expected Values

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%.

Performance Characteristics

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, unformed, unpreserved 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 the 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. 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. Out of the 984 clinical specimens included in the prospective study analysis, 956 (97.2%) generated valid ARIES® *C. difficile* Assay results on the first attempt. Another 15 specimens had to be rerun due to operator error or improper sample storage. Overall, five (5) specimens remained invalid by ARIES® *C. difficile* Assay upon retest. Overall failure rate after repeat testing was 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 the combined results of 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 84.7%. Negative percent agreement of the ARIES® *C. difficile* Assay for toxigenic *C. difficile* against direct toxigenic culture

was 92.6% (809/874, Lower Bound 95% CI, 90.6%) and specificity compared to the combination of direct and enriched toxigenic culture was 95.8% (797/832, Lower Bound 95% B CI, 94.2%).

TABLE 4. **ARIES® *C. difficile* Assay Performance Compared to Direct Culture (N=979)**

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

[a] 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.

[b] 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.

[c] 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 5. **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[b]	168
Negative	14[a]	797	811
TOTAL	147	832	979[c]
		95% CI	
Sensitivity	90.5%	84.6% - 94.2%	
Specificity	95.8%	94.2% - 97.0%	

[a] 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.

[b] 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 thattargeted genomic regions distinct from the ARIES® *C. difficile* Assay.

[c] 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.

Table 6 provides a summary of the general demographic information of the 984 prospectively collected stool specimens that were included in the prospective analysis.

TABLE 6. **General Demographic Details of the Clinical Study Population (N=984)**

	Number of Subjects	% Distribution
Gender		
Male	472	48.0%
Female	512	52.0%
Total	984	100%
Age (yrs)		
<2	1	0.1%
2 - 11	4	0.4%
12 - 21	27	2.7%
22 - 59	462	47.0%
≥60	490	49.8%
Total	984	100%
Subject Status		
Outpatients	479	48.7%
Hospitalized	482	49.0%
Emergency Department	20	2.0%
Long Term Care Facility	3	0.3%
Total	984	100%

Analytical Performance

Limit of Detection

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® *C. difficile* Assay using five representative reference strains of toxigenic *C. difficile* (BAA-1871, ATCC 43598, BAA- 1812, BAA-1803, BAA-1871). A preliminary LoD concentration was estimated by testing a six point, three-fold dilution series, of strain BAA-1871 in processed Negative Stool Matrix.

The LoD for each strain was determined by testing replicates of 20 at levels around the estimated LoD concentration. The observed LoD was determined as the lowest concentration that had a positivity rate of ≥95%. The number of CFU/cassette at LoD concentration was determined by colony counting. The final LoD concentrations are shown in *Table 7*.

TABLE 7. **Limit of Detection of the ARIES® *C. difficile* Assay**

ATCC® Number	Toxinotype	*tcdA*/*tcdB* Genes[b]	LoD Concentration		Positivity
			CFU/mL Stool	CFU/cassette	
BAA-1871	0	+/+	31.2	1.0	19/20 (95.0%)
43598	VIII	-/+	139.9	4.7	20/20 (100%)
BAA-1812	XII	+/+	110.4	3.7	20/20 (100%)
BAA-1803	IIIc [a]	+/+	18.6	0.6	20/20 (100%)
BAA-1870	IIIb [a]	+/+	19.2	0.6	20/20 (100%)

[a] Outbreak-associated Pulsed Field Gel Electrophoresis type NAP1

[b] As reported by ATCC

Interfering Substances

The effect of potential interfering substances on the ARIES® *C. difficile* Assay was evaluated by spiking prepared solutions of potential interfering substances into negative stool specimen with and without toxigenic *C. difficile* culture (BAA-1870 or BAA-1871; both *tcdA*$^+$/*tcdB*$^+$) near the device's limit of detection. Three replicates were tested in the presence of each potentially interfering substance. A total of 14 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 8. **Interfering Substance Panel**

Interfering Substance	Concentration of Interfering Substance Tested
Barium sulfate	1.3% w/v
Fecal fat (Triglycerides)	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 was 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 and Cross Contamination

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

Analytical Reactivity

Analytical reactivity performance characteristics for the ARIES® *C. difficile* Assay were assessed by testing a panel of 15 *C. difficile* strains in addition to those included in the LoD Study (13 toxinotype 0 and one each of toxinotypes V and XXII). All strains known to be positive for *tcdA* and/or *tcdB* were detected appropriately at a concentration near the limit of detection. Additionally, *in-silic*o analysis suggests that the ARIES® *C. difficile* Assay will detect toxinotype X, although this was not demonstrated functionally.

TABLE 9. **Analytical Reactivity Panel**

ATCC® Number	Strain	Toxinotype	*tcdA/tcdB* Genes [a]
BAA-1382	630	0	+/+
43255	VPI 10463	0	+/+
BAA-1875	5325	V	+/+
BAA-1874	4205	0	+/+
700792	14797-2	0	+/+
43600	2149	0	+/+
BAA-1808	NA	0	+/+
BAA-1873	5283	0	+/+
17858	1253	0	+/+
BAA-1811	NA	0	+/+
BAA-1815	NA	0	+/+
BAA-2156	LBM 0801040	0	+/+
BAA-1872	4206	0	+/+
BAA-1806	NA	0	+/+
BAA-1814	NA	XXII	+/-

[a] As reported by ATCC®.

Analytical Specificity

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, plus human DNA. The potential for cross-reactivity or interference was evaluated by testing three replicates in the presence of toxigenic *C. difficile* strains BAA-1870 and BAA-1871 at concentrations near the device's limit of detection. Each organism or virus was also tested in *C. difficile* Negative Stool Matrix. Bacteria were tested at ≥10^6 CFU/ml and viruses at ≥10^5 $TCID_{50}$/mL, or the highest available concentration. Human genomic DNA was also tested at 5 µg/mL.

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.

The potential for cross reaction or interference by *C. botulinium*, was evaluated by *in-silico* analysis. Based on this analysis, no cross-reaction or interference is expected.

TABLE 10. **Cross-Reacting and Microbial Interference Panel**

	Organism Name	Test Concentration
1	*Abiotrophia defectiva*	5.80 x 10^7 CFU/mL
2	*Acinetobacter baumannii*	1.0 x 10^6 CFU/mL[c]
3	Adenovirus Type 7A	5.12 x 10^6 $TCID_{50}$/mL
4	*Aeromonas hydrophila*	7.5 x 10^7 CFU/mL
5	*Alcaligenes faecalis subsp. faecalis*	1.1 x 10^9 CFU/mL
6	*Bacillus cereus*	4.87 x 10^6 CFU/mL[c]
7	*Bacteroides fragilis*	2.39 x 10^8 CFU/mL
8	*Campylobacter coli*	2.55 x 10^7 CFU/mL
9	*Campylobacter jejuni*	1.44 x 10^6 CFU/mL[c, e]
10	*Candida albicans*	1.33 x 10^7 CFU/mL
11	*Citrobacter freundii*	1.45 x 10^8 CFU/mL[c,d]
12	*Clostridium bifermentans*	5.25 x 10^7 CFU/mL[c]
13	*Clostridium butyricum*	2.24 x 10^7 CFU/mL
14	*Clostridium haemolyticum*	1.29 x 10^5 CFU/mL[b]
15	*Clostridium perfringens*	5.30 x 10^6 CFU/mL
16	*Clostridium scindens*	8.90 x 10^7 CFU/mL
17	*Clostridium septicum*	8.10 x 10^6 CFU/mL
18	*Clostridium sordellii*	1.64 x 10^6 CFU/mL
19	*Clostridium sporogenes*	5.15 x 10^7 CFU/mL[d]
20	*Clostriduim novyi*	1.40 x 10^8 cells/mL
21	Coxsackievirus (Type A16)	2.04 x 10^6 $TCID_{50}$/mL

	Organism Name	Test Concentration
22	Cytomegalovirus (Type AD-169)	5.74 x 10^5 $TCID_{50}$/mL
23	Echovirus Type 11	2.94 x 10^6 $TCID_{50}$/mL
24	*Edwardsiella tarda*	4.42 x 10^7 CFU/mL
25	*Enterobacter aerogenes*	8.75 x 10^8 CFU/mL
26	*Enterobacter cloacae*	2.99 x 10^8 CFU/mL
27	*Enterococcus faecalis vanB*	4.95 x 10^7 CFU/mL
28	Enterovirus (Type 71)	2.08 x 10^4 $TCID_{50}$/mL[b]
29	*Escherichia coli (026:H4)*	1.80 x 10^8 CFU/mL[c]
30	*Escherichia coli (O157:H7)*	2.05 x 10^8 CFU/mL[c]
31	*Flavonifactor plautii* [a]	3.07 x 10^7 CFU/mL
32	*Helicobacter pylori*	9.80 x 10^6 CFU/mL
33	Human genomic DNA	5 µg/mL[c, d, e]
34	*Klebsielia oxytoca*	5.20 x 10^8 CFU/mL
35	*Lactobacillus acidophilus*	1.25 x 10^7 CFU/mL
36	*Listeria monocytogenes*	4.65 x 10^8 CFU/mL
37	Non-toxigenic *Clostridium difficile* strain 43593	5.05 x 10^6 CFU/mL
38	Non-toxigenic *Clostridium difficile* strain 43601	1.01 x 10^7 CFU/mL
39	Non-toxigenic *Clostridium difficile* strain 43602	3.17 x 10^6 CFU/mL
40	Non-toxigenic *Clostridium difficile* strain 43603	4.05 x 10^6 CFU/mL
41	Norovirus Group I	4.26 x 10^6 $TCID_{50}$/mL
42	Norovirus Group II	4.26 x 10^6 $TCID_{50}$/mL
43	*Peptostreptococcus anaerobius*	2.29 x 10^6 CFU/mL
44	*Plesiomonas shigelloides*	1.52 x 10^8 CFU/mL
45	*Porphyromonas asaccharolytica*	3.70 x 10^6 CFU/mL
46	*Prevotella melaninogenica*	2.05 x 10^6 CFU/mL
47	*Proteus mirabilis*	1.42 x 10^8 CFU/mL[c]
48	*Providencia alcalifaciens*	2.07 x 10^8 CFU/mL
49	*Pseudomonas aeruginosa*	1.97 x 10^8 CFU/mL
50	Rotavirus	8.49 x 10^3 $TCID_{50}$/mL[b]

	Organism Name	Test Concentration
51	*Salmonella enterica (typhimurium)*	5.95 x 10^8 CFU/mL
52	*Salmonella enterica subsp. arizonae*	5.80 x 10^8 CFU/mL
53	*Salmonella enterica subsp. enterica*	2.60 x 10^8 CFU/mL
54	*Serratia liquefaciens*	5.45 x 10^8 CFU/mL [c]
55	*Serratia marcescens*	1.00 x 10^6 CFU/mL[c, d, e]
56	*Shigella boydii*	2.32 x 10^8 CFU/mL
57	*Shigella dysentariae*	1.59 x 10^8 CFU/mL
58	*Shigella sonnei*	1.15 x 10^8 CFU/mL
59	*Staphylococcus aureus*	5.45 x 10^8 CFU/mL
60	*Staphylococcus epidermidis*	1.45 x 10^8 CFU/mL
61	*Streptococcus agalactiae*	8.25 x 10^7 CFU/mL
62	*Vibrio parahaemolyticus*	1.07 x 10^8 CFU/mL

[a] Same species as *Clostridium orbisceindens*.

[b] Tested at the highest concentration available.

[c] Toxigenic *C. difficile* Negative Samples

A. baumannii: when tested at 4.8 x 10^7 CFU/mL, 3/3 replicates produced Invalid results; when retested at 1 x 10^6 CFU/mL, 3/3 replicates were reported as Toxigenic *C. difficile* Negative.

B. cereus, *C. jejuni*, *C. freundii*, *E. coli* O26:H4, *P. mirabilis*, *S. liquefaciens* and Human DNA: on initial testing, 1/3 replicates produced an Invalid result; upon repeat testing at the same concentration 1/1 replicates was reported as Toxigenic *C. difficile* Negative.

S. marcescens: when tested at 9.7 x 10^7 CFU/mL, 2/3 replicates produced Invalid results; when retested at 1 x 10^6 CFU/mL, 5/6 replicates were reported as Toxigenic *C. difficile* Negative and 1/6 was reported as Invalid.

C. bifermentans and *E. coli* O157:H7: on initial testing, 1/3 replicates was reported as Toxigenic *C. difficile* Positive; upon repeat testing at the same concentration 3/3 replicates were reported as Toxigenic *C. difficile* Negative.

[d] Toxigenic *C. difficile* Positive Samples containing ATCC BAA-1870

C. freundii and *C. sporogenes*: on initial testing, 1/3 replicates reported as Invalid; 1/1 replicates reported as Toxigenic *C. difficile* Negative upon repeat.

S. marcescens: when tested at 9.7 x 10^7 CFU/mL, 1/3 replicates was reported as Toxigenic *C. difficile* Negative; when retested at 1 x 10^6 CFU/mL, 3/3 replicates reported as Toxigenic *C. difficile* Positive.

Human DNA: on initial testing, 1/3 replicates was reported as Toxigenic *C. difficile.* Negative; upon repeat testing at the same concentration, 3/4 replicates were reported as Toxigenic *C. difficile* Positive and 1/4 replicates as Invalid.

[e] Toxigenic *C. difficile* Positive Samples containing ATCC BAA-1871

C. jejuni and Human DNA: on initial testing, 1/3 replicates was reported as Invalid; when retested at the same concentration 1/1 replicates was reported as Toxigenic *C. difficile.*

S. marcescens: when tested at 9.7 x 10^7 CFU/mL, 1/3 replicates was reported as Invalid; when retested at 1 x 10^6 CFU/mL, 4/4 replicates were reported as Toxigenic *C. difficile* Positive.

Reproducibility

Reproducibility of the ARIES® *C. difficile* Assay was evaluated by testing one lot of ARIES® *C. difficile* Assay cassettes on two ARIES® Systems by two operators at each of three sites on five non-consecutive days. A reproducibility panel was prepared containing a moderate positive, low positive, and high negative/ low positive independently for two *C. difficile* culture strains (BAA-1870 and BAA-1871) as well as a negative sample. The reproducibility panels were created by an independent operator and blinded. The results of the reproducibility study are in *Table 11* and *Table 12*.

TABLE 11. **ARIES® *C. difficile* Assay Site to Site Reproducibility Results**

	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[a]	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	96.7%	30/30	100%

[a] 1/30 samples was reported as Invalid on initial testing; reported Toxigenic *C. difficile* Positive upon repeat.

The expected results for the reproducibility panel targets are 100% for Moderate Positive, 95% for Low Positive, and 20% to 80% for High Negative/Low Positive for BAA-1870 and BAA-1871. The negative target is expected to be 0% positive.

TABLE 12. **ARIES® *C. difficile* Assay Reproducibility Panel Total Results**

	Agreement with expected results		95% Confidence Interval
BAA-1870 Moderate Positive	90/90	100%	95.9% to 100%
BAA-1870 Low Positive	90/90	100%	95.9% to 100%
BAA-1870 High Negative/Low Positive	76/90	84.4%	75.6% to 90.5%
BAA-1871 Moderate Positive	90/90	100%	95.9% to 100%
BAA-1871 Low Positive	89/90	98.9%	94.0% to 99.8%
BAA-1871 High Negative/Low Positive	75/90	83.3%	74.3% to 89.6%
***C. difficile* Negative**	89/90	98.9%	94.0% to 99.8%

The expected results for the reproducibility panel targets are 100% for Moderate Positive, 95% for Low Positive, and 20% to 80% for High Negative/Low Positive for BAA-1870 and BAA-1871. The negative target is expected to be 0% positive.

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 for assessing

repeatability. A repeatability panel was prepared containing moderate positive, low positive and high negative/low positive sample independently for two *C. difficile* culture strains as well as a negative sample. The results of the repeatability study are in *Table 13*.

TABLE 13. **ARIES® *C. difficile* Assay Within Laboratory Precision/Reproducibility Results**

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

[a] 2/36 samples produced Invalid results on initial testing; both reported as Positive upon repeat

The expected results for the reproducibility panel targets are positive for Moderate Positive (100%), Low Positive (95%), and High Negative/Low Positive (20% to 80%) for BAA-1870 and BAA-1871. The negative target is expected to be negative (100%).

References

CDC - *Clostridium Difficile* Infection-Healthcare-associated Infections. February 25, 2015. Atlanta (GA): Centers for Disease Control and Prevention. [accessed 2015 October]. *http://www.cdc.gov/HAI/organisms/cdiff/Cdiff_infect.html*

Monot, M. et al. *Clostridium difficile*: *New Insights into the Evolution of the Pathogenicity Locus*. Scientific Reports 5, Article number: 15023 (2015) doi:10.1038/srep15023

McFarland L.V. *Emerging therapies for Clostridium difficile infections*. Expert Opin Emerg Drugs 2011; 16: 425–39.

O' Brien J.A., Lahue B.J., Caro J.J., et al. *The emerging infectious challenge of Clostridium difficile associated disease in Massachusetts hospitals: clinical and economic consequences*. Infect Control Hosp Epidemiol 2007; 28: 1219–27.

Keller PM, Weber MH. Rational Therapy of *Clostridium difficile* Infections.*Viszeralmedizin*. 2014;30(5):304-309. doi:10.1159/000366302.

Sambol S.P., Merrigan M.M., Lyerly D., Gerding D.N., and Johnson *S. Toxin Gene Analysis of a Variant Strain of Clostridium difficile That Causes Human Clinical Disease*. Barbieri J.T., ed. Infection and Immunity. 2000;68(10):5480-5487.

Sloan L.M., Duresko B.J., Gustafson D.R., Rosenblatt J.E. *Comparison of Real-Time PCR for Detection of the tcdC Gene with Four Toxin Immunoassays and Culture in Diagnosis of Clostridium difficile Infection*. Journal of Clinical Microbiology. 2008;46(6):1996-2001. doi:10.1128/JCM.00032-08.

Surawicz C.M., et al. *Guidelines for Diagnosis, Treatment, and Prevention of Clostridium difficile Infections*. Am J Gastroenterol 2013; 108:478–498.

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