Luminex

Package Insert | IVD

ARIES® Norovirus Assay

IVD For *In Vitro* Diagnostic Use.

For Use With ARIES® Systems.

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ARIES® Norovirus Assay Package Insert

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ARIES® Norovirus Assay: 50-10042
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CE

EC REP

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Luminex Corporation (Luminex) reserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with applicable regulatory requirements. Luminex assumes no liability for any damages resulting from the off-label application or misuse of this information.

This product, or use thereof, is covered, in whole or in part, or made by processes covered by one or more patents: *www.luminexcorp.com/patents*.

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.	5.1.7* 	Serial number Indicates the manufacturer's serial number so that a specific medical device can be identified.

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)
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.	5.5.1* IVD	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

* ANSI/AAMI/ISO 15223-1:2012, Medical devices—Symbols to be used with medical devices 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)

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

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Intended Use

The ARIES® Norovirus Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the identification and differentiation of norovirus genogroup I (GI) and genogroup II (GII) RNA from raw or unpreserved unformed stool specimens collected from individuals with symptoms of acute gastroenteritis.

The ARIES® Norovirus Assay is intended to aid in the diagnosis of norovirus infections when used in conjunction with clinical evaluation, laboratory findings, and epidemiological information. The assay also aids in the detection and identification of norovirus infections in the context of outbreaks.

The ARIES® Norovirus Assay is indicated for use with ARIES® Systems.

Summary and Explanation of the Test

Noroviruses are single stranded RNA viruses in the genus Norovirus, family Caliciviridae (Zheng). Noroviruses are also known as “winter-vomiting disease” or “stomach-flu” due to their spread in human population especially during the winter months. Primarily spread through the fecal-oral route, noroviruses are highly contagious and 10-100 viral particles may be sufficient to infect an individual (ECDC - Factsheet).

Norovirus causes inflammation of the stomach and/or the intestines. The most common symptoms include diarrhea, vomiting, nausea and stomach pain. Most infections are self limiting and a majority of the patients get better within 1 - 3 days (CDC - Symptoms). However, severe outcomes and longer duration of illness may be reported among the elderly. (CDC - Norovirus in health care facilities fact sheet)

Traditionally, Enzyme Immunoassays (EIA’s) have been used for detection of norovirus antigen in clinical samples. However, most EIA’s offer modest performance when compared to real time PCR tests. The sensitivity of EIA’s when compared to PCR based tests ranges from 36% to 80% and specificity ranges from 47% to 100%. (CDC-MMWR Recommendations and Reports)

The ARIES® Norovirus 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. The ARIES® Norovirus Assay detects and differentiates norovirus genogroup I and norovirus genogroup II.

Principles of the Procedure

Unpreserved fresh or frozen raw stool sample is processed using the ARIES® Stool Resuspension Buffer and the provided ARIES® Norovirus Assay Loops. ARIES® Stool Resuspension Buffer is added directly to the ARIES® Norovirus Assay cassette sample chamber. Primary stool sample is added to the sample chamber of an ARIES® Norovirus Assay cassette containing ARIES® Stool Resuspension Buffer using the ARIES® Norovirus Assay Loop.

The cassette is then placed into the ARIES® magazine which can hold up to six cassettes. The magazine is inserted into an ARIES® instrument. A barcode on top of the ARIES® Norovirus Assay cassette is automatically scanned by the ARIES® instrument, associating a preloaded ARIES® Norovirus Assay protocol file with the cassette. The ARIES® Norovirus 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 sample lysis, 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® Norovirus Assay cassette. Purified nucleic acids are automatically transferred to the cassette’s PCR tube that contains the lyophilized Norovirus Master Mix for the PCR amplification step. The Norovirus Master Mix contains primer pairs and probes specific to norovirus genogroup I and norovirus genogroup II, and the SPC sequence. Total assay time, including extraction and PCR cycling, takes approximately 2 hours.

Materials Provided

The ARIES® Norovirus Assay Complete Kit (Part Number 50-10042) includes 24 assay cassettes and a pack of 25 ARIES® Norovirus Assay Loops.

The assay protocol file, package insert, and *ARIES® Quick Guide* ship separately on a USB as part of the ARIES® Norovirus Assay Protocol File Kit (CN-0338-01).

TABLE 1. **ARIES® Norovirus Contents Provided by Luminex**

Item	Number	Description
ARIES® Norovirus Assay Complete Kit	50-10042	24 ARIES® Norovirus Assay cassettes which contain necessary reagents for sample extraction, nucleic acid purification, and amplification. A 25 pack of ARIES® Norovirus Assay Loops.
ARIES® Norovirus Assay Protocol File Kit	CN-0338-01	An assay protocol file, a package insert, and an *ARIES® Quick Guide* containing instructions for use, are provided on a USB.

Materials Required But Not Provided

Reagents:

- ARIES® Stool Resuspension Buffer (30-00103)

NOTE: Each bottle of the ARIES® Stool Resuspension Buffer has enough volume to run about 50 samples.

Equipment:

- Appropriately sized pipettor
- 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
- Vortex mixer

Plasticware and Consumables

- Nuclease-free aerosol-barrier pipette tips

Warnings and Precautions

1. For *In Vitro* Diagnostic Use.
2. 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*.
3. Thoroughly clean and disinfect all surfaces with 10% bleach.
4. Avoid contamination from positive controls and samples by following good laboratory practices.
5. Avoid contamination by using a new nuclease-free aerosol barrier tip to add ARIES® Stool Resuspension Buffer.
6. The ARIES® Norovirus Assay Loops are single-use. Do not reuse the ARIES® Norovirus Assay Loops.
7. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.

8. Follow your institution's safety procedures for working with chemicals and handling biological samples.
9. Do not use cassettes, kits, or reagents beyond their expiration date.
10. The cassettes are single-use. Do not reuse cassettes.
11. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
12. Only use the assay protocol file provided by Luminex on the USB drive.
13. Mucin at cassette input concentrations of 0.044% w/v and above may cause interference and may result in false negatives.
14. Only use the procedures described in this package insert. Any deviation from the outlined procedures can result in assay failure or cause erroneous results.
15. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
16. ARIES® cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheet (SDS) regarding safe handling practices for any spills.
17. 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.
18. Refer to the appropriate ARIES® system operation manual for electrical and mechanical warnings.
19. Do not let the ARIES® Systems get wet or allow standing water to pool under the instrument.
20. 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 resuspend, and continue testing. The ARIES® Stool Resuspension Buffer must be ordered separately using part number 30-00103.

ARIES® Norovirus Assay cassettes are shipped refrigerated. Store at room temperature (15°C to 30°C) after receipt.

Always check the expiration date on the kit box, the cassettes, and the ARIES® Stool Resuspension Buffer.

Sample Collection, 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 norovirus 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) or frozen state (≤ -70°C), as appropriate.

Sample Storage

Samples can be stored at room temperature up to 24 hours, refrigerated at 2°C to 8°C for up to 5 days, or frozen at -65°C to -95°C for up to 1 month from the date of collection.

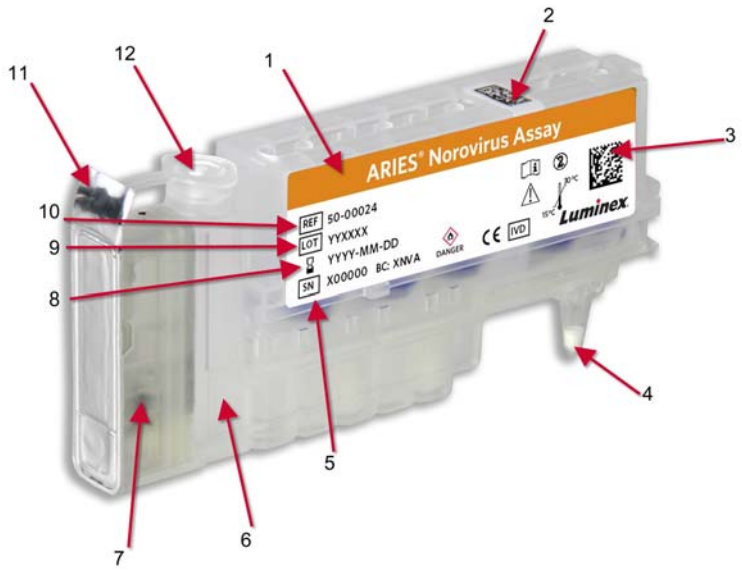
Software Setup

Importing Assay Files to ARIES® Systems

The ARIES® Norovirus 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 Import Assay 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

Adding Samples to the 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. Obtain stool sample(s), the ARIES® Norovirus Assay cassettes, the ARIES® Norovirus Assay Loop, and the ARIES® Stool Resuspension Buffer. If necessary, thaw the stool sample(s).
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. Vortex the sample for 5 to 10 seconds to homogenize the mixture.
7. Open the cassette cap to access the cassette sample chamber.
8. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 400 µL of ARIES® Stool Resuspension Buffer. Insert the pipette tip near the bottom of the cassette sample chamber and expel the ARIES® Stool Resuspension buffer.

   CAUTION: Ensure the correct volume of buffer is used.

9. Using the ARIES® Norovirus Assay Loop, remove stool sample from the sample container.

   CAUTION: Ensure the correct amounts of sample are used.

   NOTE: Completely fill the ARIES® Norovirus Assay Loop with stool, but do not let it overflow.

   Fill the ARIES® Norovirus Assay Loop with stool as pictured:

   NOTE: The pictures below are accurate representations of the appropriate color of the ARIES® Norovirus Assay Loop to be used.

   This picture displays too little stool on the ARIES® Norovirus Assay Loop:

This picture displays too much stool on the ARIES® Norovirus Assay Loop:

When using liquid stool, use the amount of liquid stool pictured below:

10. Place the ARIES® Norovirus Assay Loop containing the stool sample into the cassette sample chamber by inserting the loop to the bottom of the chamber making sure the loop contacts the ARIES® Stool Resuspension Buffer. Gently swirl the loop in the ARIES® Stool Resuspension Buffer to transfer the stool sample to the cassette and ensure stool resuspension. Discard the ARIES® Norovirus Assay Loop.

11. Close the cassette cap to seal the cassette sample chamber.

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

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 New Order 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.

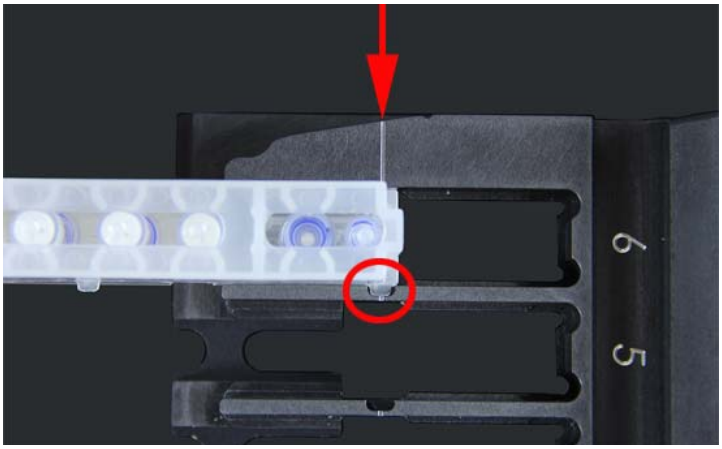
a. If applicable, to add a control, choose **Control** in the **Sample Type** drop-down menu.

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. Pick up and scan the Sample ID on the sample tube or enter the required information manually.
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 the 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® Norovirus Assay cassettes, and 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, start the run manually by highlighting the module you want, then selecting 

from the Page Action bar.

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 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 melting temperature value provided in the assay protocol file. All assay outcomes are listed in *Table 2*. Norovirus positivity is based on detection of the norovirus genogroup I and/or norovirus genogroup II target.

TABLE 2. **Interpretation of Results**

Example	SPC		Norovirus Genogroup I		Norovirus Genogroup II		Norovirus Call
	Ct value	Melt	Ct value	Melt	Ct value	Melt	
1	N/A	N/A	+	+	+	+	Norovirus GI Positive Norovirus GII Positive
2	N/A	N/A	N/A	-	+	+	Norovirus GI Negative Norovirus GII Positive
3	N/A	N/A	+	+	N/A	-	Norovirus GI Positive Norovirus GII Negative
4	+	+	N/A	-	N/A	-	Norovirus GI Negative Norovirus GII Negative
5	N/A	-	N/A	-	N/A	-	Invalid
6	-	+	N/A	-	N/A	-	Invalid
7	N/A	N/A	N/A	N/A	-	+	Invalid
8	N/A	N/A	-	+	N/A	N/A	Invalid

Legend	
+	Indicates that the value is detected within the appropriate parameters
-	Indicates that the value is not detected within the appropriate parameters
N/A	Not applicable. All outcomes will result in the same call.

Invalid Results

In case of an “Invalid Result”, re-test the sample with a new assay cassette. If the problem is unresolved, contact Luminex Technical Support.

Quality Control

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

TABLE 3. **Control to Monitor Quality**

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

Each ARIES® Norovirus 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.

Limitations

1. The detection of viral 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. There is a risk of false negative results due to the presence of sequence variants in the targets of the assay, procedural errors, amplification inhibitors in samples, or inadequate numbers of organism(s) for amplification.
4. 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.
5. Cross-reactivity with organisms other than those tested can lead to erroneous results.
6. This test cannot rule out diseases caused by other pathogens.
7. 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.

Performance Characteristics

Clinical Performance

Two hundred (200) norovirus positive or norovirus negative raw, unpreserved stool specimens were retrospectively collected from six (6) geographically diverse sites in the United States and EU. The specimens were tested with one replicate by two operators using the ARIES® Norovirus Assay to assess performance with clinical stool specimens. The specimens were reference tested using a CE-IVD Nucleic Acid Amplification Test (NAAT) reference method. The performance of ARIES® Norovirus Assay when compared to the NAAT reference method is summarized in *Table 4* and *Table 5*.

TABLE 4. **Summary of Norovirus GI Results (N=200)**

ARIES® Norovirus Assay	NAAT Reference Method		
	Norovirus GI Positive	Norovirus GI Negative	Total
Norovirus GI Positive	14	1	15
Norovirus GI Negative	1	184	185
Total	15	185	200
		95% CI	
PPA	93.3%	70.2% - 98.8%	
NPA	99.5%	97.0% - 99.9%	

TABLE 5. **Summary of Norovirus GII Results (N=200)**

ARIES® Norovirus Assay	NAAT Reference Method		
	Norovirus GII Positive	Norovirus GII Negative	Total
Norovirus GII Positive	84	1	85
Norovirus GII Negative	1	114	115
Total	85	115	200
		95% CI	
PPA	98.8%	93.6% - 99.8%	
NPA	99.1%	95.2% - 99.8%	

Table 6 and *Table 7* represent post-discordant analysis performed using bi-directional sequencing analysis with analytically validated primers that used a different primer sequence from those used in the ARIES® Norovirus Assay.

TABLE 6. **Summary of Norovirus GI Results after Discordant Analysis (N = 200)**

ARIES® Norovirus Assay	NAAT Reference Method and Bi-directional Sequencing		
	Norovirus GI Positive	Norovirus GI Negative	Total
Norovirus GI Positive	14	1	15
Norovirus GI Negative	0	185	185
Total	14	186	200
		95% CI	
PPA	100%	78.5% - 100%	
NPA	99.5%	97.0% - 99.9%	

TABLE 7. **Summary of Norovirus GII Results after Discordant Analysis (N = 200)**

ARIES® Norovirus Assay	NAAT Reference Method and Bi-directional Sequencing		
	Norovirus GII Positive	Norovirus GII Negative	TOTAL
Norovirus GII Positive	84	1	85
Norovirus GII Negative	0	115	115
TOTAL	84	116	200
		95% CI	
PPA	100%	95.6% - 100%	
NPA	99.1%	95.3% - 99.8%	

Analytical Performance

Limit of Detection

A Limit of Detection (LoD) study was performed to determine the analytical sensitivity of the ARIES® Norovirus Assay using three (3) norovirus GI (norovirus GI.3) well characterized stool samples and two (2) norovirus GII (norovirus GII.4) well characterized stool samples.

The LoD concentrations were established in a preliminary study and were confirmed with the well characterized Norovirus stool samples diluted to concentrations one dilution at and below the LoD concentrations and tested with twenty four (24) replicates. The RNA copies / mL was determined for each specimen IDs dilutions using a validated real time reverse transcriptase polymerase chain reaction method.

The final assay LoD claim is 3.1E+04 copies/mL for norovirus GI and 4.4E+03 copies/mL for norovirus GII.

Interfering Substances

The effect of potential interfering substances on the ARIES® Norovirus Assay was evaluated by testing three replicates of each 3X LoD norovirus GI specimen, 3X LoD norovirus GII specimen, and Negative samples (Negative Stool Matrix) spiked with 14 potential interfering substances. At the listed cassette input concentrations of the substances, the substances do not interfere with the assay. All norovirus positive results were 100% positive and all norovirus negative results were 100% negative. However, Mucin at the cassette input concentration of 0.044% or above showed interference and resulted in false negatives for both the norovirus GI and norovirus GII.

TABLE 8. **Interfering Substances**

Interfering Substance	Cassette Input Concentration
Whole Blood	0.5% v/v
Preparation H®	50.0% w/v
Mucin	0.0044% w/v[1]
Fecal fat - Triglycerides	4.8% w/v
Fecal fat - Cholesterol	0.06% w/v
Amoxicillin	7.5 µmol/L
Acetaminophen	1362 µmol/L
Pepto-Bismol™ (Active Ingredient: Bismuth subsalicylate)	0.063% w/v
Kaopectate® (Active Ingredient: Attapulgite)	0.064 mg/mL
IMODIUM®	5.0% w/v
Ibuprofen	2,436 µmol/L
Aspartame	5.1% w/v
Metronidazole	709 µmol/L
Barium sulfate	0.016% w/v

[1] Mucin at the cassette input concentration of 0.044% w/v and above caused interference and resulted in false negatives for both the norovirus GI and norovirus GII results.

Co-infection Verification

A study was designed to evaluate the ability of the ARIES® Norovirus Assay to detect norovirus GI and norovirus GII analytes when both are present in one specimen. Analytes were tested at high (200X LoD) and low concentrations (3X LoD) using 12 replicates. The ARIES® Norovirus Assay is able to detect co-

infections where one analyte is present near the LoD and the other is present at a high concentration, as well as co-infections where both analytes are present at a high concentration.

TABLE 9. **Co-infection Results**

Norovirus Condition	Result	Percent of Replicates
GI High / GII Low	Norovirus GI Positive, Norovirus GII Positive	100% (12/12)
GI Low / GII High	Norovirus GI Positive, Norovirus GII Positive	100% (12/12)
GI High / GII High	Norovirus GI Positive, Norovirus GII Positive	100% (12/12)

Carry-Over and Cross Contamination

Carry-over and cross contamination for the ARIES® Norovirus Assay was assessed by testing thirty (30) high positive norovirus GII samples and thirty (30) norovirus negative samples (Negative Stool Matrix). Samples were tested in an alternating pattern with high positive samples run adjacent to negative samples across five (5) consecutive runs. No carry-over and cross contamination was observed. The overall percent agreement was 100% for positive and negative samples.

Analytical Inclusivity

An analytical reactivity study was performed to functionally evaluate the analytical inclusivity of the ARIES® Norovirus Assay using twenty-three (23) norovirus strains: 8 norovirus GI and 15 norovirus GII. Previously typed norovirus specimens were used as the source for the functionally tested norovirus strains. The RNA copies/mL was determined for each specimen using a validated real time reverse transcriptase polymerase chain reaction method. Testing with the ARIES® Norovirus Assay consisted of 6 replicates per strain. The results from functional testing are summarized in the table below.

An *in-silico* analysis was also performed for norovirus strains, including strains that were unavailable for functional testing. In addition to the strains that were functionally tested, the following strains are expected to be detected based on the *in-silico* results: GI.P3-GI.3, GII.P16-GII.2, GII.P2-GII.2, GII.P21-GII.3, GII.4, GII.4 (2006b Minerva), GII.Pe-GII.4 2012, GII.P7-GII.6, GII.9, GII.10, GII.P21-GII.13, GII.P17-GII.17, and GII.21

TABLE 10. **Analytical Inclusivity of Norovirus GI**

Inclusivity Strains	Detected Concentration (copies/mL)
GI.1	8.3E+02
GI.2	9.2E+02
GI.3	9.3E+02
GI.4	9.0E+02
GI.5	8.9E+02
GI.6	8.5E+02
GI.7	9.1E+02
GI.8	9.3E+02

TABLE 11. **Analytical Inclusivity of Norovirus GII**

Inclusivity Strains	Detected Concentration (copies/mL)
GII.1	2.2E+02

GII.2	2.2E+02
GII.3	4.2E+03
GII.4 New Orleans	4.4E+03
GII.4 Sydney	4.4E+03
GII.5	4.4E+03
GII.6	4.3E+03
GII.7	4.0E+03
GII.8	4.4E+03
GII.12	4.3E+03
GII.13	2.1E+04
GII.14	4.4E+03
GII.15	2.2E+04
GII.16	4.2E+03
GII.17	2.1E+04

Analytical Specificity

A study was performed to evaluate cross reactivity and interference of the ARIES® Norovirus Assay with fifty-eight (58) microorganisms that might be present in stool specimens. The effect of potential cross reactivity or interference was evaluated by testing 3 replicates of each 3X LoD norovirus GI specimen, 3X LoD norovirus GII specimen, and Negative replicates (Negative Stool Matrix) spiked with 58 potential cross reacting organisms. All organisms were tested at clinically relevant concentrations or higher. At the listed cassette input concentrations of the organisms, the organisms do not cross react or interfere with the assay: all norovirus positive results were 100% positive and all norovirus negative results were 100% negative.

TABLE 12. **Analytical Specificity**

Cross Reacting Organism	Cassette Input Concentration
Acinetobacter baumannii	1.0 x 10^6 cfu/mL
Acinetobacter lwoffii	1.0 x 10^6 cfu/mL
Adenovirus	1.0 x 10^5 $TCID_{50}$/mL
Aeromonas caviae complex	1.0 x 10^6 cfu/mL
Aeromonas hydrophila complex	1.0 x 10^6 cfu/mL
Astrovirus	4.2 x 10^3 $TCID_{50}$/mL
Bacillus cereus toxin	1.0 x 10^6 cfu/mL
Blastocystis hominis (Genomic DNA)	0.5 µg/mL
Campylobacter coli	1.0 x 10^6 cfu/mL
Campylobacter jejuni	1.0 x 10^6 cfu/mL
Candida albicans	1.0 x 10^6 cfu/mL

Citrobacter freundii	1.0 x 10^6 cfu/mL
Clostridium difficile toxin A/B producers	1.0 x 10^6 cfu/mL
Clostridium sordellii	1.0 x 10^6 cfu/mL
Coxsackie	1.0 x 10^5 $TCID_{50}$/mL
Cryptosporidium parvum	1.0 x 10^6 oocysts/mL
Echovirus	1.0 x 10^5 $TCID_{50}$/mL
Entamoeba histolytica	N/A[1]
Enterobacter cloacae	1.0 x 10^6 cfu/mL
Enterococcus faecalis	1.0 x 10^6 cfu/mL
Enterococcus faecium	1.0 x 10^6 cfu/mL
E. coli O157:H7	1.0 x 10^6 cfu/mL
E. coli O26:H11	1.0 x 10^6 cfu/mL
E. coli O45:H2	1.0 x 10^6 cfu/mL
E. coli O103:H11	1.0 x 10^6 cfu/mL
E. coli O111	1.0 x 10^6 cfu/mL
E. coli O121	1.0 x 10^6 cfu/mL
E. coli O145	1.0 x 10^6 cfu/mL
Escherichia hermannii	1.0 x 10^6 cfu/mL
Giardia lamblia	1.0 x 10^6 cfu/mL
Helicobacter pylori	1.0 x 10^6 cfu/mL
Lactococcus lactis	1.0 x 10^6 cfu/mL
Listeria monocytogenes	1.0 x 10^6 cfu/mL
Morganella morganii	1.0 x 10^6 cfu/mL
Plesiomonas shigelloides	1.0 x 10^6 cfu/mL
Proteus mirabilis	1.0 x 10^6 cfu/mL
Proteus vulgaris	1.0 x 10^6 cfu/mL
Providencia stuartii	1.0 x 10^6 cfu/mL
Pseudomonas aeruginosa	1.0 x 10^6 cfu/mL
Pseudomonas fluorescens	1.0 x 10^6 cfu/mL
Pseudomonas putida	1.0 x 10^6 cfu/mL
Rotavirus	1.0 x 10^5 $TCID_{50}$/mL

Salmonella agona	1.0 x 10^6 cfu/mL
Salmonella bongori	1.0 x 10^6 cfu/mL
Salmonella enterica	1.0 x 10^6 cfu/mL
Sapovirus (Synthetic RNA)	4.8 x 10^3 copies/µL
Serratia proteamaculans	1.0 x 10^6 cfu/mL
Shigatoxin STX1	1 µg/mL
Shigatoxin STX2	1 µg/mL
Shigella flexneri	1.0 x 10^6 cfu/mL
Shigella sonnei	1.0 x 10^6 cfu/mL
Staphylococcus aureus	1.0 x 10^6 cfu/mL
Streptococcus agalactiae	1.0 x 10^6 cfu/mL
Streptococcus dysgalactiae	1.0 x 10^6 cfu/mL
Vibrio cholerae	1.0 x 10^6 cfu/mL
Vibrio parahaemolyticus	1.0 x 10^6 cfu/mL
Streptococcus mutans (Viridans Steptococci)	1.0 x 10^6 cfu/mL
Yersinia enterocolitica	1.0 x 10^6 cfu/mL

[1] Concentration information not available.

Reproducibility

Reproducibility of the ARIES® Norovirus Assay was evaluated by testing one lot of ARIES® Norovirus Assay Cassettes on two ARIES® instruments by two operators at each of three sites on at least five non-consecutive days. A reproducibility panel was prepared containing a moderate positive (approximately 10X LoD for both norovirus GI and norovirus GII) and low positive (approximately 1X LoD for both norovirus GI and norovirus GII) independently for norovirus GI and norovirus GII as well as a negative. The reproducibility panels were created by an independent operator and blinded. The results of the reproducibility study are presented in the table below.

TABLE 13. **Reproducibility Panel Results**

	Site 1		Site 2		Site 3		Total Agreement with expected results[1]		95% Confidence Interval
	Agreement with expected results[1]		Agreement with expected results[1]		Agreement with expected results[1]				
Norovirus GI Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96.0–100%
Norovirus GI Low Positive	30/30	100%	30/30	100%	29/30	96.7%	89/90	98.9%	94.0–100%[2]
Norovirus GII Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96.0–100%
Norovirus GII Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96.0–100%
Norovirus Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96.0–100%

[1]The expected results for:

Norovirus GI Moderate Positive target was 100% Norovirus GI Positive, Norovirus GII Negative;

Norovirus GI Low Positive was approximately 95% Norovirus GI Positive, Norovirus GII Negative;

Norovirus GII Moderate Positive target was 100% Norovirus GI Negative, Norovirus GII Positive;

Norovirus GII Low Positive was approximately 95% Norovirus GI Negative, Norovirus GII Positive;

Norovirus Negative was 100% Norovirus GI Negative, Norovirus GII Negative.

[2] Upper limit of 95% C.I. is 99.97%, rounded to 100%.

Precision

Within Laboratory Precision/Repeatability of the ARIES® Norovirus Assay was evaluated by two operators performing testing across multiple ARIES® instruments using one lot of ARIES® Norovirus Assay Cassettes. Testing was performed for at least 5 days per operator and included a total of 300 replicates used in assessing repeatability. A reproducibility panel was prepared containing moderate positive (approximately 10X LoD for both norovirus GI and norovirus GII) and low positive (approximately 1X LoD for both norovirus GI and norovirus GII) samples independently for norovirus GI and norovirus GII as well as a negative sample. The results of the repeatability study are shown in *Table 14*.

TABLE 14. **Repeatability Panel Results**

Target Type	Agreement with Expected Results[1]	95% Confidence Interval
Norovirus GI Moderate Positive	100% (60/60)	94.0 – 100%
Norovirus GI Low Positive	96.7% (58/60)	88.5 – 99.6%
Norovirus GII Moderate Positive	100% (60/60)	94.0 – 100%
Norovirus GII Low Positive	100% (60/60)	94.0 – 100%

Norovirus Negative	100% (60/60)	94.0 – 100%

[1]The expected results for:

Norovirus GI Moderate Positive target was 100% Norovirus GI Positive, Norovirus GII Negative;

Norovirus GI Low Positive was approximately 95% Norovirus GI Positive, Norovirus GII Negative;

Norovirus GII Moderate Positive target was 100% Norovirus GI Negative, Norovirus GII Positive;

Norovirus GII Low Positive was approximately 95% Norovirus GI Negative, Norovirus GII Positive;

Norovirus Negative was 100% Norovirus GI Negative, Norovirus GII Negative.

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