Luminex

Package Insert | IVD

ARIES® HSV 1&2 Assay

IVD For *In Vitro* Diagnostic Use.

For Use With ARIES® Systems.

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ARIES® HSV 1&2 Assay Package Insert

89-30000-00-534 Rev. F
Assay Kit Part Number: 50-10031
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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.

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 Risks Indicates that there are potential biological risks associated with the medical device.
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* 	Authorized representative in the European Community Indicates the Authorized representative in the European Community.	‡ 	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking
5.1.7* 	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 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)

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Intended Use

The ARIES® HSV 1&2 Assay (EU) is a real-time polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection and typing of herpes simplex virus (HSV 1&2) DNA in cutaneous or mucocutaneous lesion specimens from symptomatic patients or in CSF from patients suspected of HSV infections of the central nervous system (CNS). The test is indicated for use with symptomatic individuals to aid in the diagnosis of HSV infections.

Negative CSF results do not preclude HSV 1 and HSV 2 infection and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

The assay is not intended to be used for prenatal screening.

The ARIES® HSV 1&2 Assay (EU) is indicated for use with ARIES® Systems.

Summary and Explanation of the Test

HSV 1 and HSV 2 are common human pathogens that cause infections in neonates, children and adults worldwide. Combined, HSV infections affect 40 million people in the United States and cause 600,000 new infections every year (Nadelman and Newcomer 2000). Following primary infection, these viruses can establish latency in the dorsal root ganglia of the infected host, and can cause re-occurring lesions when the virus travels through nerve cells to either oral or genital sites. HSV 1 is generally associated with infection in the tongue, mouth, lips, pharynx and eyes, whereas HSV 2 is primarily associated with genital and neonate infection. HSV transmission can result from direct contact with secretions from either a symptomatic or asymptomatic host (Tronstein, Johnston, et al. 2011). Though there is no cure, infected individuals can manage viral re-emergence with various drug therapies (Modi, Van et al. 2008 and Superti, Ammendolia et al. 2008).

In addition to oral and genital infection, HSV 1&2 can cause infections of the central nervous system. Meningitis is an inflammation of the tissue that covers the brain and spinal cord. Viral meningitis is the most common type of meningitis. Infants younger than 1 month old and people with weakened immune systems are more likely to have severe illness (CDC 2015). Due to the nature of the infection, obtaining results from cerebrospinal fluid (CSF) in a timely fashion is critical.

Viral isolation, direct or indirect fluorescent antibody testing, in situ hybridization, and serology can be used to diagnose HSV infections (Jerome and Morrow 2011). However, due to length of culture time, sample transport difficulties, procedural complexity, and lack of desirable sensitivity, nucleic acid amplification methods such as PCR are often preferred as the diagnostic test method (Filén, Strand et al. 2004 and Slomka 2000).

The ARIES® HSV 1&2 Assay uses Luminex Corporation's 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® HSV 1&2 Assay detects and differentiates HSV 1 and HSV 2 DNA sequences using thermal melt (T_m) analysis.

Principles of the Procedure

Primary sample is added directly to the ARIES® HSV 1&2 Assay cassette sample chamber. 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® HSV 1&2 Assay cassette is automatically scanned by the ARIES® instrument, associating a preloaded ARIES® HSV 1&2 Assay protocol file with the cassette. The ARIES® HSV 1&2 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, the 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® HSV 1&2 Assay cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized HSV 1&2 Master Mix for the PCR amplification step. The lyophilized HSV 1&2 Master Mix

contains a primer pair specific to HSV 1 and HSV 2, and a second primer pair specific to the SPC sequence. Total assay time, including extraction and PCR cycling, takes approximately two hours.

Materials Provided

The ARIES® HSV 1&2 Assay (Part Number 50-10031) contains 24 assay cassettes.

The assay protocol file, package insert, and *ARIES® Quick Guide* ship separately on a USB as part of the ARIES® HSV 1&2 Assay Protocol File Kit (CN-0371-01).

TABLE 1. **ARIES® HSV 1&2 Assay Contents Provided By Luminex**

Item	Part Number	Description
ARIES® HSV 1&2 Assay Kit	50-10031	24 ARIES® HSV 1&2 Assay cassettes which contain the necessary reagents for sample extraction, nucleic acid purification, and amplification.
ARIES® HSV 1&2 Assay Protocol File Kit	CN-0371-01	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 for cutaneous or mucocutaneous specimen collection:

- Copan® Universal Viral Transport Medium (Copan UTM)

Equipment:

- -70°C to -80°C freezer
- 2°C to 8°C refrigerator
- 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
- Appropriately sized pipettor

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 an individual primary specimen aliquot to each cassette.
6. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.

7. Follow your institution's safety procedures for working with chemicals and handling biological samples.
8. Do not use cassettes, kits, or reagents beyond their expiration date.
9. The cassettes are single-use. Do not reuse cassettes.
10. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
11. Only use the extraction protocol file provided by Luminex on the USB drive.
12. Only use the procedures described in this package insert. Any deviation from the outlined procedures can result in extraction failure or cause erroneous results.
13. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
14. ARIES® cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheet (SDS) regarding safe handling practices for any spills.
15. 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.
16. Refer to the appropriate ARIES® system operation manual for electrical warnings.
17. Do not let ARIES® Systems get wet or allow standing water to pool under the instruments.
18. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Reagent Storage, Handling, and Stability

ARIES® HSV 1&2 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 and cassettes.

Specimen Handling and Storage

Specimen Collection

Cutaneous and mucocutaneous lesion swab and CSF specimens should be obtained by appropriately trained individuals.

Specimen Transport

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

Transport specimens refrigerated at 2°C to 8°C. If there will be a long delay before specimen processing (greater than 15 days from the date of collection), specimens should be frozen at -70°C or colder and transported on dry ice.

Specimen Storage

Specimens can be stored refrigerated at 2°C to 8°C for up to 15 days from the date of collection. If specimens will be used after 15 days from the date of collection, store frozen at ≤ -70°C (Wiedbrauk and Cunningham 1996).

Store left-over specimens at -70°C or colder.

Assay Procedure

Adding Assay Files to ARIES® Systems

The ARIES® HSV 1&2 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**.

Entering Orders

Sample barcodes are scanned to associate them with an order. An assay cassette is also then scanned to specify the assay and associate the cassette with a specific sample. Refer to *"Running an Assay"* on page 8 for more information.

The Sample ID is required on all orders and is the link between sample and cassette. The Accession ID and Requisition Number can also perform this role and associate the cassette to the sample, but are optional unless otherwise chosen to be required by the user. You can set requirement options in the Sample Options dialog box located on the Order Management Settings page.

Enabling the Automatic Print and Export Results Options

The Auto Print and Auto Export options are settings that need to be enabled prior to starting the run on the ARIES® instrument. Results can also be printed and exported manually after a run. Refer to *"Manually Printing Reports"* on page 9 for more information.

To enable the Auto Print feature, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Results > Settings**.
2. Toggle the **Generate Reports After Run** button to **Yes.**
3. For the **Sample Reports to Printer** option, select **Default** or **All**.

To export results automatically after a run, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Results > Settings**.
2. Toggle the **Summary Report as CSV** button to **Yes.**
3. Enter the **CSV Output Location** by selecting the folder icon in the upper right-hand corner of the **CSV Output Location** box. The **Select Folder** dialog box opens.
   a. Choose the **Location** for the export.
   b. Select **OK**.

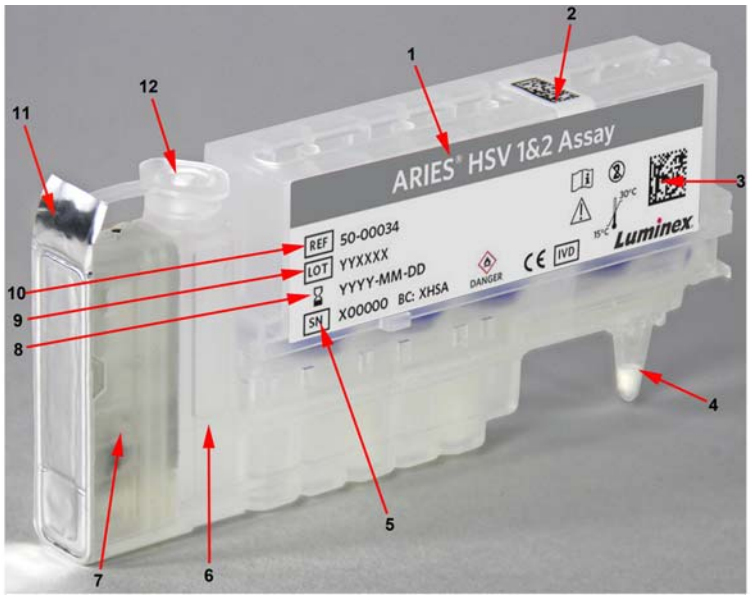
To automatically export LIS results as either HL7 or CSV, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Administration > LIS Settings.**
2. Toggle the **Enable Export to LIS** button to **Yes**.
3. Toggle the **Auto Export to LIS** button to **Yes.**
4. Enter the **LIS Export Location** by selecting the folder icon in the upper right-hand corner of the **LIS Export Location** box. The **Select Folder** dialog box opens.

a. Choose the **Location** for the export.
b. Select **OK**.

CAUTION: Validation of LIS compatibility must be performed by the user.

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

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. 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.

4. Close the cassette cap to seal the cassette sample chamber.
5. 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, toggle 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. Refer to the appropriate ARIES® system operation manual for more information on controls.

6. Pick up and scan the Sample ID on the sample tube or enter the required information manually.
7. Scan the **Data Matrix** barcode on the screen next to **Save**, or manually select **Save**.

Adding Samples to the Cassettes

1. Place the sample tube in the Sample Prep Tray.
2. 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.

3. Place the cassette in the Sample Prep Tray next to the sample.

4. Vortex the primary sample for 5 to 10 seconds to homogenize the mixture.
5. Open the cassette cap to access the cassette sample chamber.
6. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 200 µL of sample from the sample tube.

CAUTION: Ensure that the correct amounts of sample are used.

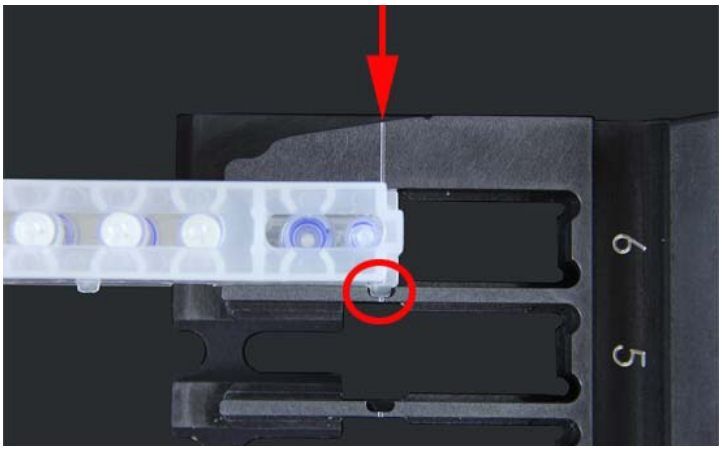
CAUTION: Use care to avoid contamination of the pipettor during transfer of the sample from the sample tube to the cassette.

7. Place the sample in the cassette sample chamber by inserting the pipette tip near the bottom of the chamber before expelling the sample.

8. 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.

9. 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.

10. Gently insert the cassette into the magazine.
11. 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® HSV 1&2 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 Start Run 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 Status 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.

Reviewing, Printing, and Exporting Run Results

When the ARIES® HSV 1&2 Assay run finishes successfully, the cassettes are colored green on the Run page. See *Table 2* for other color indicators. Refer to the appropriate ARIES® system operation manual for more color definitions.

TABLE 2. **Color Indicators**

Color	Reason
Red	Cassettes contain errors, were not scanned successfully, require additional information, or the run failed or was aborted. Contact Luminex Technical Support for assistance.
Yellow	Information was manually entered on the Run page or the cassette is expired.
Green	Run finished successfully, the cassettes were scanned with no errors.
Blue	Magazine is inserted and a cassette is detected for this slot.
Purple	Module is currently running, the magazine slot is in use.
White	Empty module, no magazine is inserted, or no cassette detected.

The Run page includes visual indicators such as a status bar, an estimated time to completion indicator, and a Run Complete notification once the run has completed.

Automatically Printing and Exporting Results

NOTE: To ensure that the LIS Reporting (Auto Print) feature is enabled, check that Sample Reports to Printer is set to All or Default in the Export Settings dialog box located on the Results > Settings page.

When the run finishes successfully, the result reports are automatically printed at the default printer and exported in .csv and .pdf format to the designated location. Refer to *"Enabling the Automatic Print and Export Results Options"* on page 4.

Manually Printing Reports

To manually select a report to print, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Results**. Regardless of the type of report you want to view, select only one result. Otherwise, the **Create Report** icon grays out.
2. Select **Create Report** from the Page Action bar. Choose the type of report you want to view from the drop-down menu. There are three options: **Run Report**, **Detailed Report,** and **Summary Report**.

   NOTE: Selecting a single result gives you the option to generate a **Run Report**, **Summary Report**, or a **Detailed Report**. You cannot select more than one result and run a **Summary Report** or a **Detailed Report** -- the **Create Report** icon grays out. When generating a **Run Report**, you can select multiple results from the same run and still use the **Create Report** icon. With **Run Report**, the **Create Report** icon is disabled only when results from multiple runs are selected. Once the report opens, choose to export 

   or print the result 

   .

   - The Run Report displays the run results for all samples in the run and any comments or logs associated with that run.
   - The Summary Report displays the run result for one individual sample and any comments or logs associated with that sample.

- The Detailed Report displays all cassette information for one individual sample, the Amplification and Melt Graphs, and any comments or logs associated with that sample. Access to run this report is restricted to users with administrative rights.

Manually Exporting Results

To manually export results, complete the following:

1. Select the result(s) to export on the **Results** page and select 

from the Page Action bar.
2. Choose the **Location** and the **File Name** for the export in the **Export File** dialog box and select **OK**.

NOTE: You can only export to a USB drive or a mapped network drive.

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 melting temperature (T_m) value provided in the assay protocol file. All assay outcomes are listed in *Table 3*.

TABLE 3. **Interpretation of Sample Results**

Example	SPC		HSV			Call
	Ct Value	T_m Value	Ct Value	HSV 1 T_m Value	HSV 2 T_m Value	
1	N/A	+	+	+	+	HSV 1&2 Positive
2	N/A	+	+	+	-	HSV 1 Positive
3	N/A	+	+	-	+	HSV 2 Positive
4	+	+	-	-	-	HSV 1&2 Negative
5	+	+	>[a]	N/A	N/A	
6	-	+	>[a] / -	N/A	N/A	Invalid
7	N/A	-	N/A	N/A	N/A	
8	N/A	+	-	+	N/A	
9	N/A	+	-	N/A	+	

N/A: Not applicable. All possible outcomes will result in the same call.

[a] Greater than the Ct cut-off value.

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 4*.

TABLE 4. **Controls 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® HSV 1&2 Assay cassette contains a sample processing control, which is processed with the sample and analyzed during the amplification reaction.

Control strains may be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference HSV 1 and HSV 2 strain or well characterized HSV 1 and HSV 2 clinical isolates may be used as positive controls. Universal Viral Transport Medium may be used as a negative control.

External controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

Limitations

1. Negative CSF results do not preclude HSV 1 and HSV 2 infection and should not be used as the sole basis for treatment and other patient management decisions.
2. The ARIES® HSV 1&2 Assay should not be used for prenatal screening.
3. 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.
4. There is a risk of false negative results due to improperly collected, transported, or handled samples.
5. 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.
6. 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.
7. The ARIES® HSV 1&2 Assay may not detect a co-infection of HSV 1 and HSV 2 in specimens where the two virus types are not equally represented in clinical specimens.
8. The ARIES® HSV 1&2 Assay detects and differentiates between HSV 1 and HSV 2 only. It does not detect or differentiate any other Herpes virus types.
9. The ARIES® HSV 1&2 Assay does not distinguish between infectious and non-infectious HSV 1 and HSV 2.
10. Results from the ARIES® HSV 1&2 Assay should be interpreted in conjunction with other clinical and laboratory findings.
11. The ARIES® HSV 1&2 Assay is for use with swab specimens collected and stored in Copan Universal Viral Transport Medium and CSF. Performance characteristics of other specimen types have not been established.
12. For use only on 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 - Mucocutaneous and Cutaneous

The performance of the ARIES® HSV 1&2 Assay was assessed at three (3) geographically diverse clinical sites in the United States. A total of 1963 left-over clinical specimens from symptomatic male and female patients were included in the clinical study. Of these, 1500 specimens were prospectively collected (all comers). The remaining 463 were pre-selected for cutaneous and mucocutaneous lesion types that were under-represented in the initial prospective sample set. All of the pre-selected specimens were also prospectively collected. Of the 1963 specimens tested, fifty-five (55) specimens were lesion sources from anatomical sites that could not be determined, four (4) specimens remained invalid upon re-testing by the ARIES® HSV 1&2 Assay and three (3) were unavailable for re-testing. All of these 62 specimens were excluded from accuracy determinations.

The reference/comparative method used to evaluate the clinical performance of ARIES® HSV 1&2 Assay was ELVIS® HSV-ID and D^3 Typing Test System. Because the ELVIS® method provides no information on HSV 1 patient infected status (positive or negative) in specimens that test positive for HSV 2, all specimens that were positive for HSV 2 by the ELVIS® HSV-ID and D^3 Typing System were excluded from the analysis of HSV 1 clinical performance. A total of 448 cutaneous lesions specimens were tested. One hundred and one (101) specimens that were positive for HSV 2 by the ELVIS® HSV-ID and D^3 Typing

System were excluded from the analysis of HSV 1 clinical performance. A total of 1453 mucocutaneous lesions specimens were tested. Two hundred and sixty three (263) specimens that were positive for HSV 2 by the ELVIS® HSV-ID and D^3 Typing System were excluded from the analysis of HSV 1 clinical performance. The performance of ARIES® HSV 1&2 Assay when compared to ELVIS® viral culture is summarized for cutaneous and mucocutaneous lesions in the tables below.

TABLE 5. **Summary of HSV 1 Results for Cutaneous Lesions (N=347)**

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	**Negative**	**TOTAL**
Positive	51	17[a]	68
Negative	5[b]	274	279
TOTAL	56	291	347
		95% CI	
Sensitivity	91.1% (51/56)	80.4% - 97.0%	
Specificity	94.2% (274/291)	90.8% - 96.6%	

[a] Thirteen (13) HSV 1 ARIES® positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. The remaining four (4) false positive specimens were negative for both HSV 1 and HSV 2 by bi-directional sequencing.

[b] All five (5) HSV 1 ARIES® negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. One of these specimens was positive for HSV 2 by both ARIES® and sequencing.

TABLE 6. **Summary of HSV 1 Results for Mucocutaneous Lesions (N=1190)**

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	**Negative**	**TOTAL**
Positive	262	42[a]	304
Negative	8[b]	878	886
TOTAL	270	920	1190
		95% CI	
Sensitivity	97.0% (262/270)	94.2% - 98.7%	
Specificity	95.4% (878/920)	93.9% - 96.7%	

[a] Nineteen (19) HSV 1 ARIES® positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Twenty (20) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing. The remaining three (3) specimens were unavailable (QNS) for sequence analysis.

[b] Seven (7) HSV 1 ARIES® negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. One of these specimens was positive for HSV 2 by both ARIES® and sequencing. One (1) false negative specimen was confirmed as positive for HSV-1 by bi-directional sequencing.

TABLE 7. **Summary of HSV 2 Results for Cutaneous Lesions (N=448)**

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	96	39[a]	135
Negative	5[b]	308	313
TOTAL	101	347	448
		95% CI	
Sensitivity	95.0% (96/101)	88.8% - 98.4%	
Specificity	88.8% (308/347)	85.0% - 91.9%	

[a] Thirty five (35) HSV 2 ARIES® positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. The remaining four (4) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing.

[b] All five (5) HSV 2 ARIES® negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Two of these specimens were positive for HSV 1 by both ARIES® and sequencing.

TABLE 8. **Summary of HSV 2 Results for Mucocutaneous Lesions (N=1453)**

ARIES® HSV 1&2 Assay	Reference Method		
	Positive	Negative	TOTAL
Positive	259	81[a]	340
Negative	4[b]	1109	1113
TOTAL	263	1190	1453
		95% CI	
Sensitivity	98.5% (250/263)	96.2% - 99.6%	
Specificity	93.2% (1109/1190)	91.6% - 94.6%	

[a] Fifty eight (58) HSV 2 ARIES® positive specimens that were negative by the reference method were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Twenty-one (21) false positive specimens were negative for both HSV-1 and HSV-2 by bi-directional sequencing. The remaining two (2) specimens were unavailable (QNS) for sequence analysis.

[b] All four (4) HSV 2 ARIES® negative specimens that were positive by the reference method were negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® HSV 1&2 Assay. Three (3) of these specimens were positive for HSV 1 by both ARIES® and sequencing.

Clinical Performance - CSF

Ninety-eight (98) HSV CSF specimens were tested in one to three replicates by two operators using the ARIES® HSV 1&2 Assay to assess performance with CSF clinical specimens. The specimens were reference tested using a Nucleic Acid Test (NAT) reference method. Due to low prevalence of HSV Positive CSF specimens, both natural and contrived positive specimens were used. Contrived positive specimens were made by independently diluting HSV Positive viral culture stock into individual HSV Negative CSF specimens to clinically relevant concentrations. All natural specimens were tested by both the NAT Reference Method and an alternate NAT reference method.

TABLE 9. **2x2 Contingency Table for HSV 1 - CSF Specimens (N = 188)**

ARIES® HSV 1&2 Assay	NAT Reference Method		
	Positive	**Negative**	**Total**
Positive	14	19[a]	33
Negative	0	155	155
Total	14	174	188
		95% CI	
PPA	100.0%	78.5% – 100.0%	
NPA	89.1%	83.6% – 92.9%	

[a] Nineteen (19) replicates of HSV 1 ARIES® positive specimens that were negative by the NAT reference method were positive. One of the 19 specimens was positive by an alternate NAT reference method. All of the remaining specimens were individually contrived from diluted viral culture stock in negative CSF specimens to clinically relevant positive concentrations.

TABLE 10. **2x2 Contingency Table For HSV 2 - CSF Specimens (N = 180)**

ARIES® HSV 1&2 Assay	NAT Reference Method		
	Positive	**Negative**	**Total**
Positive	6	33[a]	39
Negative	0	141	141
Total	6	174	180
		95% CI	
PPA	100.0%	61.0% – 100.0%	
NPA	81.0%	74.6% – 86.2%	

[a] Thirty-two (32) replicates of HSV 2 ARIES® Positive specimens that were negative by the NAT reference method were positive. Nine of the 32 specimens were positive by an alternate NAT reference method. All of the remaining specimens were individually contrived from diluted viral culture stock in negative CSF specimens to clinically relevant positive concentrations.

The following tables represent post-discordant analysis, where contrived positive specimens and natural specimens that tested positive by both ARIES® and the alternate NAT reference method are positive.

TABLE 11. **2x2 Contingency Table for HSV 1 - CSF Specimens (N = 188)**

ARIES® HSV 1&2 Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	33	0	33
Negative	0	155	155
Total	33	155	188
		95% CI	
PPA	100.0%	89.6% to 100.0%	
NPA	100.0%	97.6% to 100.0%	

TABLE 12. **2x2 Contingency Table For HSV 2 - CSF Specimens (N = 180)**

ARIES® HSV 1&2 Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	38	1	39
Negative	0	141	141
Total	38	142	180
		95% CI	
PPA	100.0%	90.8% to 100.0%	
NPA	99.3%	96.1% to 99.9%	

Analytical Performance

Limit of Detection

A Limit of Detection (LoD) study was performed at Luminex Corporation to evaluate the analytical sensitivity of the ARIES® HSV 1&2 Assay using two representative strains of HSV 1 (MacIntyre and F) and two representative strains of HSV 2 (MS & G). Preliminary LoD concentrations were determined by performing a six-point, five-fold dilution series in Copan® Universal Transport Media, of each quantified ($TCID_{50}$/mL) strain. The observed LoD of a HSV strain was determined as the lowest concentration that had a positivity rate of ≥ 95%.

The LoD concentrations determined in the preliminary study were confirmed with the same HSV 1 and HSV 2 reference strains diluted to the preliminary LoD concentrations and tested with twenty-four (24) replicates. The final LoDs are presented in *Table 13*.

TABLE 13. **Limit of Detection of the ARIES® HSV 1&2 Assay**

HSV Type	Strain	LoD Concentration ($TCID_{50}$/mL)	Positivity
HSV 1	MacIntyre	7.11E+03	24/24 (100.0%)
	F	16.5	23/24 (95.8%)
HSV 2	MS	2.7	24/24 (100.0%)
	G	2.8	24/24 (100.0%)

The final assay LoD claim is 7.11E+03 $TCID_{50}$/mL for HSV 1 and 2.7 $TCID_{50}$/mL for HSV 2 in both Copan® Universal Transport Media and CSF. The final assay LoD claims for Universal Transport Media and CSF are equivalent.

Co-infection Verification

A study was designed to evaluate the ability of the ARIES® HSV 1&2 Assay to detect HSV 1 and HSV 2 analytes when both are present in one specimen. Analytes were tested at high (200X LoD) and low concentrations (5X LoD) using 12 replicates. The ARIES® HSV 1&2 Assay may not detect a co-infection of HSV 1 and HSV 2 in cases where the two virus types are not equally represented in clinical specimens (*Table 14*). Co-infections were only detected when both analytes were present at 200X LoD. An HSV analyte at 5X LoD was not detected in the presence of a different HSV analyte at 200X LoD.

TABLE 14. **Co-infection Results**

Condition	Result	Percent of Replicates
HSV 1 High / HSV 2 Low	HSV 1 Positive	100.0% (12/12)
HSV 2 High / HSV 1 Low	HSV 2 Positive	100.0% (12/12)
HSV 1 and HSV 2 High	HSV 1&2 Positive	100.0% (12/12)

Interfering Substances

The effect of potential interfering substances on the ARIES® HSV 1&2 Assay was evaluated by testing five replicates of each 5X LoD HSV 1 viral culture, 5X LoD HSV 2 viral culture, and Negative samples (Copan® UTM) spiked with 30 potential interfering substances. At the tested concentrations of the substances, the substances do not interfere with the assay. All HSV positive results were 100% positive and all HSV 1&2 Negative results were 100% negative.

TABLE 15. **Interfering Substance Results**

Interfering Substance	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Abreva® (Docosanol)	10%	100%	100%	0%
Acyclovir (Acycloguanosine)	2.5 mg/mL	100%	100%	0%
Buffy Coat	5%	100%	100%	0%
Carmex® Cold Sore Lip Balm	1%	100%	100%	0%
Casein	7.0 mg/mL	100%	100%	0%
Clotrimazole 3 Vaginal Cream	1%	100%	100%	0%
Toothpaste	5%	100%	100%	0%
Anti-itch cream (Benzalkonium Chloride)	5%	100%	100%	0%
Cidofovir	2.5 mg/mL	100%	100%	0%
Douche	10%	100%	100%	0%
Foscarnet	2.5 mg/mL	100%	100%	0%
Ganciclovir	2.5 mg/mL	100%	100%	0%
Valganciclovir	2.5 mg/mL	100%	100%	0%
Leukocytes	10%	100%	100%	0%
Lip Clear Lysine+®	1%	100%	100%	0%

Interfering Substance	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Listerine®	10%	100%	100%	0%
Male Urine	10%	100%	100%	0%
Female Urine	10%	100%	100%	0%
Whole Blood	10%	100%	100%	0%
Monistat® 1	5%	100%	100%	0%
Monistat® 3	5%	100%	100%	0%
Albumin	10 mg/mL	100%	100%	0%
Releev® Cold Sore Treatment	1%	100%	100%	0%
K-Y® Brand Jelly	5%	100%	100%	0%
Spermicide	5%	100%	100%	0%
Tioconazole	5%	100%	100%	0%
Vagisil® Cream	1%	100%	100%	0%
Yeast Gard®	1%	100%	100%	0%
Hemoglobin	0.625 mg/mL	100%	100%	0%
Topical antiseptic (Betadine)	5% v/v	100%	100%	0%

Analytical Specificity

A study was performed to evaluate cross reactivity and interference of the ARIES® HSV 1&2 Assay with seventy-six (76) microorganisms that might be present in cerebrospinal fluid (CSF) or cutaneous or mucocutaneous lesion specimens. The effect of potential cross reactivity or interference was evaluated by testing 5 replicates of each 5X LoD HSV 1 viral culture, 5X LoD HSV 2 viral culture, and Negative replicates (Copan® UTM) spiked with 76 potential cross reacting organisms. All organisms were tested at the highest available concentration. At the tested concentrations of the organisms, the organisms do not cross react or interfere with the assay: all HSV positive results were 100% positive and all HSV 1&2 Negative results were 100% negative (*Table 16*).

TABLE 16. **Analytical Specificity Results**

Cross-Reacting Organisms	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Acinetobacter calcoaceticus	9.3×10^7 cfu/mL	100%	100%	0%
Bacteroides fragilis	4.2×10^8 cfu/mL	100%	100%	0%
Candida albicans	1.7×10^7 cfu/mL	100%	100%	0%
Candida glabrata	7.9×10^6 cfu/mL	100%	100%	0%
Chlamydia trachomatis	1.8×10^4 $TCID_{50}$/mL	100%	100%	0%
Clostridium sordellii	4.9×10^6 cfu/mL	100%	100%	0%
Cytomegalovirus (AD169 Strain)	1.2×10^6 $TCID_{50}$/mL	100%	100%	0%

Cross-Reacting Organisms	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Enterobacter cloacae	7.4×10^8 cfu/mL	100%	100%	0%
Enterococcus faecalis	4.6×10^8 cfu/mL	100%	100%	0%
Enterovirus (Type 71)	4.2×10^4 $TCID_{50}$/mL	100%	100%	0%
Epstein-Barr virus (B95-8 Strain)	9.3×10^7 cpy/mL	100%	100%	0%
Escherichia coli	5.1×10^8 cfu/mL	100%	100%	0%
Gardnerella vaginalis	5.4×10^6 cfu/mL	100%	100%	0%
Hepatitis A Virus	8.5×10^2 IU/mL	100%	100%	0%
Hepatitis B Virus	5.6×10^8 IU/mL	100%	100%	0%
HIV-1	1.1×10^5 $TCID_{50}$/mL	100%	100%	0%
Human Herpes 6 virus (Z29 Strain)	4.2×10^4 $TCID_{50}$/mL	100%	100%	0%
Human Herpes 7 virus (SB Strain)	1.2×10^6 $TCID_{50}$/mL	100%	100%	0%
Human Papilloma virus	1.7×10^9 cpy/mL	100%	100%	0%
Lactobacillus acidophilus	2.0×10^7 cfu/mL	100%	100%	0%
Legionella micdadei	2.7×10^8 cfu/mL	100%	100%	0%
Mobiluncus mulieris	3.2×10^8 cfu/mL	100%	100%	0%
Moraxella cartarrhalis	9.9×10^5 cfu/mL	100%	100%	0%
Mycoplasma hominis	3.6×10^6 cfu/mL	100%	100%	0%
Mycoplasma orale	1.4×10^8 cfu/mL	100%	100%	0%
Mycoplasma salivarium	4.7×10^6 cfu/mL	100%	100%	0%
Neisseria gonorrhoeae	5.7×10^7 cfu/mL	100%	100%	0%
Propionibacterium acnes	3.7×10^8 cfu/mL	100%	100%	0%
Proteus mirabilis	2.1×10^8 cfu /mL	100%	100%	0%
Rubella virus	1.3×10^5 $TCID_{50}$/mL	100%	100%	0%
Salmonella enteritidis	2.1×10^7 cfu/mL	100%	100%	0%
Serratia marcescens	4.1×10^8 cfu/mL	100%	100%	0%
Staphylococcus aureus	1.4×10^9 cfu/mL	100%	100%	0%
Staphylococcus epidermidis	3.5×10^8 cfu/mL	100%	100%	0%
Streptococcus pyogenes	2.6×10^8 cfu/mL	100%	100%	0%
Staphylococcus saprophyticus	6.6×10^6 cfu/mL	100%	100%	0%

Cross-Reacting Organisms	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Streptococcus agalactiae	8.7 x 10^7 cfu/mL	100%	100%	0%
Toxoplasma gondii	6.6 x 10^5 tachyzoites/mL	100%	100%	0%
Treponema pallidum	9.8 x 10^6 genome cpy/ mL	100%	100%	0%
Trichomonas vaginalis	4.2 x 10^5 trophozoites/ mL	100%	100%	0%
Varicella Zoster virus	2.5 x 10^4 $TCID_{50}$/mL	100%	100%	0%
Acinetobacter Iwoffi	8.3 x 10^7 cfu/mL	100%	100%	0%
Haemophilus influenza type B	5.3 x 10^7 cfu/mL	100%	100%	0%
Klebsiella pneumoniae	6.3 x 10^8 cfu/mL	100%	100%	0%
Neisseria meningitides Serogroup A	7.1 x 10^8 cfu/mL	100%	100%	0%
Prevotella melaninogenica	4.1 x 10^6 cfu/mL	100%	100%	0%
Streptococcus mitis	5.7 x 10^7 cfu/mL	100%	100%	0%
Streptococcus mutans	4.4 x 10^8 cfu/mL	100%	100%	0%
Streptococcus pneumoniae	9.2 x 10^7 cfu/mL	100%	100%	0%
Streptococcus salivarius	7.5 x 10^7 cfu/mL	100%	100%	0%
Candida parapsilosis	2.9 x 10^6 cfu/mL	100%	100%	0%
Candida tropicalis	2.2 x 10^6 cfu/mL	100%	100%	0%
Human genomic DNA	10 µg/mL	100%	100%	0%
Adenovirus 2	5.0 x 10^5 U/mL	100%	100%	0%
Candida guilliermondii Z008	1.8 x 10^7 cfu/mL	100%	100%	0%
Candida krusei Z009	6.3 x 10^6 cfu/mL	100%	100%	0%
Candida lusitaniae Z010	1.4 x 10^8 cfu/mL	100%	100%	0%
Fusobacterium nucleatum	N/A[a]	100%	100%	0%
Haemophilus ducreyi	2.1 x 10^6 cfu/mL	100%	100%	0%
Mobiluncus curtisii ATCC 43063	>10^3 cfu/mL	100%	100%	0%
Simian Virus type 40 Pa-57 ATCC strain VR-239	2.8 x 10^6 $TCID_{50}$/mL	100%	100%	0%
Adenovirus 7 A	1.0 x 10^7 $TCID_{50}$/mL	100%	100%	0%
Cryptococcus neoformans	1.55 x 10^6 cfu/mL	100%	100%	0%

Cross-Reacting Organisms	Test Concentration	HSV 1 Positivity	HSV 2 Positivity	HSV 1&2 Negative Positivity
Influenza A/California/7/2009 NYMC x-179-A	1.4 x 10^4 $TCID_{50/}$mL	100%	100%	0%
Influenza B/Florida/02/2006	1.4 x 10^4 $TCID_{50/}$mL	100%	100%	0%
Listeria monocytogenes	9.30 x 10^8 cfu/mL	100%	100%	0%
Measles	5.0 x 10^6 $TCID_{50/}$mL	100%	100%	0%
Mumps virus	5.0 x 10^6 $TCID_{50/}$mL	100%	100%	0%
Mycobacterium tuberculosis (Genomic DNA)	0.6 µg/mL	100%	100%	0%
Naegleria fowleri (Genomic DNA)	0.13 µg/mL	100%	100%	0%
Parainfluenza Virus 2	5.0 x 10^4 $TCID_{50/}$mL	100%	100%	0%
Parainfluenza Virus 3	1.2 x 10^6 $TCID_{50/}$mL	100%	100%	0%
Pseudomonas aeruginosa	3.9 x 10^9 cfu/mL	100%	100%	0%
St. Louis Encephalitis (heat inactivated)	5.4 x 10^7 $TCID_{50/}$mL	100%	100%	0%
Streptococcus pneumoniae Z022; 19F	8.7 x 10^6 cfu/mL	100%	100%	0%
West Nile Virus (heat inactivated)	1.2 x 10^{11} $TCID_{50/}$mL	100%	100%	0%

[a] Concentration information not available.

Reproducibility

Reproducibility of the ARIES® HSV 1&2 Assay was evaluated by testing one lot of ARIES® HSV 1&2 Assay Cassettes on two ARIES® instruments by two operators at each of three sites on five non-consecutive days. A reproducibility panel was prepared containing a moderate positive (approximately 4X LoD for both HSV 1 and HSV 2), low positive (approximately 1X LoD for both HSV 1 and HSV 2) and high negative (approximately 0.1X LoD for HSV 1 and 0.4X LoD for HSV 2) independently for HSV 1 and HSV 2 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 *Table 17*.

TABLE 17. **Reproducibility Panel Results**

	Site 1				Site 2				Site 3				Total Agreement with expected results	95% Confidence Interval
	Agreement with expected results*	Avg T_m	% CV T_m	Avg T_m Deflection†	Agreement with expected results*	Avg T_m	% CV T_m	Avg T_m Deflection†	Agreement with expected results*	Avg T_m	% CV T_m	Avg T_m Deflection†		
HSV-1 Moderate Positive	30/30	85.5	0.16%	2.25E+06	30/30	85.5	0.12%	2.56E+06	30/30	85.6	0.18%	2.72E+06	90/90(100%)	96.0-100%
HSV-1 Low Positive	30/30	85.5	0.16%	2.04E+06	30/30	85.6	0.16%	2.24E+06	30/30	85.5	0.16%	2.45E+06	90/90(100%)	96.0-100%
HSV-1 High Negative	11/30	85.4	0.17%	1.39E+06	9/30	85.5	0.20%	2.33E+06	9/30	85.5	0.17%	2.06E+06	29/90(32.2%)	22.8-42.9%
HSV-2 Moderate Positive	30/30	87.9	0.17%	2.17E+06	30/30	87.8	0.16%	2.52E+06	30/30	87.8	0.15%	2.43E+06	90/90(100%)	96.0-100%
HSV-2 Low Positive	30/30	87.8	0.11%	1.95E+06	29/30	87.7	0.17%	2.23E+06	30/31	87.7	0.16%	2.04E+06	89/91(97.8%)	92.3-99.7%
HSV-2 High Negative	30/30	87.7	0.19%	1.75E+06	30/30	87.7	0.14%	1.98E+06	23/30	87.7	0.15%	1.94E+06	83/90(92.2%)	84.6-96.8%
HSV1&2 Negative	30/30	76.4	0.30%	2.76E+05	30/30	76.3	0.24%	3.11E+05	30/30	76.3	0.68%	3.34E+05	90/90(100%)	96.0-100%

* Agreement with expected results for the HSV 1&2 negative reflects SPC positivity since no HSV 1 or HSV 2 was detected. Expected result for HSV 1 Moderate Positive target was 100% HSV 1 Positive, HSV 1 Low Positive was approximately 95% HSV 1 Positive, HSV 1 High Negative was 20% to 80% HSV 1 Positive, HSV 2 Moderate Positive target was 100% HSV 2 Positive, HSV 2 Low Positive was approximately 95% HSV 2 Positive, HSV 2 High Negative was 20% to 80% HSV 2 Positive, and HSV 1&2 Negative was 100% HSV 1&2 Negative.

† Average T_m deflection (RFU) was calculated using all of the positive replicates for that target type. Average T_m deflection for the HSV 1&2 Negative reflects SPC T_m deflection since no HSV 1 or HSV 2 was detected.

Precision

Within Laboratory Precision/Repeatability of the ARIES® HSV 1&2 Assay was evaluated by two operators performing testing across multiple ARIES® instruments using one lot of ARIES® HSV 1&2 Assay Cassettes. Testing was performed in 10 days and included a total of 216 replicates used in assessing repeatability. A reproducibility panel was prepared containing moderate positive (approximately 4X LoD for both HSV 1 and HSV 2), low positive (approximately 1X LoD for both HSV 1 and HSV 2) and high negative (approximately 0.1X LoD for HSV 1 and 0.4X LoD for HSV 2) samples independently for HSV 1 and HSV 2 as well as a negative sample. The results of the repeatability study are shown in *Table 18*.

TABLE 18. **Repeatability Panel Results**[a]

Target Type	Agreement with Expected Results[b]	95% Confidence Interval	Average T_m	% Coefficient of Variation - T_m	Average T_m Deflection[c]
HSV 1 Moderate Positive	100.0% (72/72)	95.0% – 100.0%	85.6	0.17%	3.28E+06
HSV 1 Low Positive	100.0% (72/72)	95.0% – 100.0%	85.6	0.13%	2.88E+06
HSV 1 High Negative	45.80% (33/72)	34.0% – 58.0%	85.4	0.12%	2.18E+06
HSV 2 Moderate Positive	100.0% (72/72)	95.0% – 100.0%	87.9	0.16%	3.16E+06
HSV 2 Low Positive	100.0% (72/72)	95.0% – 100.0%	87.8	0.15%	2.75E+06
HSV 2 High Negative	97.40% (76/78)	91.0% – 99.7%	87.8	0.17%	2.39E+06
HSV 1&2 Negative	100.0% (72/72)	95.0% – 100.0%	76.5	0.66%	4.41E+05

[a] An overall invalid rate of 0.8% (4/514) was observed.

[b] Expected result for HSV 1 Moderate Positive target was 100% HSV 1 Positive, HSV 1 Low Positive was approximately 95% HSV 1 Positive, HSV 1 High Negative was 20% to 80% HSV 1 Positive, HSV 2 Moderate Positive target was 100% HSV 2 Positive, HSV 2 Low Positive was approximately 95% HSV 2 Positive, HSV 2 High Negative was 20% to 80% HSV 2 Positive, and HSV 1&2 Negative was 100% HSV 1&2 Negative.

[c] Average T_m deflection (RFU) was calculated using all of the positive replicates for that target type. Average T_m deflection for the HSV 1&2 Negative reflects SPC T_m deflection since no HSV 1 or HSV 2 was detected.

Carry-Over and Cross Contamination

Carry-over and cross contamination for the ARIES® HSV 1&2 Assay was assessed by testing fifteen (15) high positive HSV 1 samples, 15 high positive HSV 2 samples and thirty (30) HSV negative samples (Copan® UTM). Samples were tested in an alternating pattern with high positive samples run adjacent to negative samples across ten (10) consecutive runs. No carry-over and cross contamination was observed. The overall percent agreement was 100% for positive and negative samples.

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