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HSV 1&2 ELITe MGB® Assay

Reagents for DNA Real-Time PCR Amplification

REF M800584 IVD Rx only -20°C

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INTENDED USE

The HSV 1&2 ELITe MGB® Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection and differentiation of Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2) DNA in cutaneous or mucocutaneous lesion swab specimens from patients with signs and symptoms of HSV-1 or HSV-2 infection. This test is an aid in the differential diagnosis of HSV-1 and HSV-2 infections.

The HSV 1&2 ELITe MGB Assay is not FDA cleared for use with cerebrospinal fluid (CSF) specimens. The assay is not intended to be used for prenatal screening or for screening blood or blood products.

BACKGROUND

Herpes simplex virus (HSV) is a ubiquitous pathogen that causes a variety of clinical manifestations. HSV belongs to the alpha herpesvirus group. It is a non-enveloped virus that is approximately 160 nm in diameter with a linear, double-stranded DNA genome. Two types exist: herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). The overall sequence homology between HSV-1 and HSV-2 is about 50%. Both types are closely related but differ in epidemiology. HSV-1 is traditionally associated with orofacial disease, while HSV-2 is traditionally associated with genital disease [1]. Lesion location, however, is not necessarily indicative of viral type, as HSV-1 is associated with genital infections more often than HSV-2 in some unique subpopulations.

The term herpes is derived from the Greek word “to creep or crawl” and dates to early Greek civilization, approximately 2,000 years ago, in reference to the spreading nature of herpetic skin lesions. Up to 80% of herpes simplex infections are asymptomatic.

The prevalence of HSV infection worldwide has increased over the last several decades, making it a major public health concern. Prompt recognition of herpes simplex infection and early initiation of therapy are of utmost importance in the management of the genital and oral herpes [2].

ASSAY PRINCIPLE

The HSV 1&2 ELITe MGB Assay on the ELITe InGenius sample-to-result instrument performs automated nucleic acid extraction, amplification, and detection of up to twelve samples per session. The nucleic acid is isolated and purified from cutaneous and mucocutaneous lesion swab specimens and combined with the HSV 1&2 ELITe MGB Assay Monoreagent. The purified nucleic acid is subsequently amplified on the ELITe InGenius instrument in a real-time polymerase chain reaction (PCR) using the HSV 1&2 ELITe MGB Assay reagents.

The HSV 1&2 ELITe MGB Assay has been developed for qualitative PCR detection of HSV-1 and HSV-2 virus DNA.

The HSV-1 primers and probe target the glycoprotein D encoding gene, and the HSV-2 primers and probe target the glycoprotein G encoding gene.

Both HSV targets are designed to be detected in the presence of an internal control.

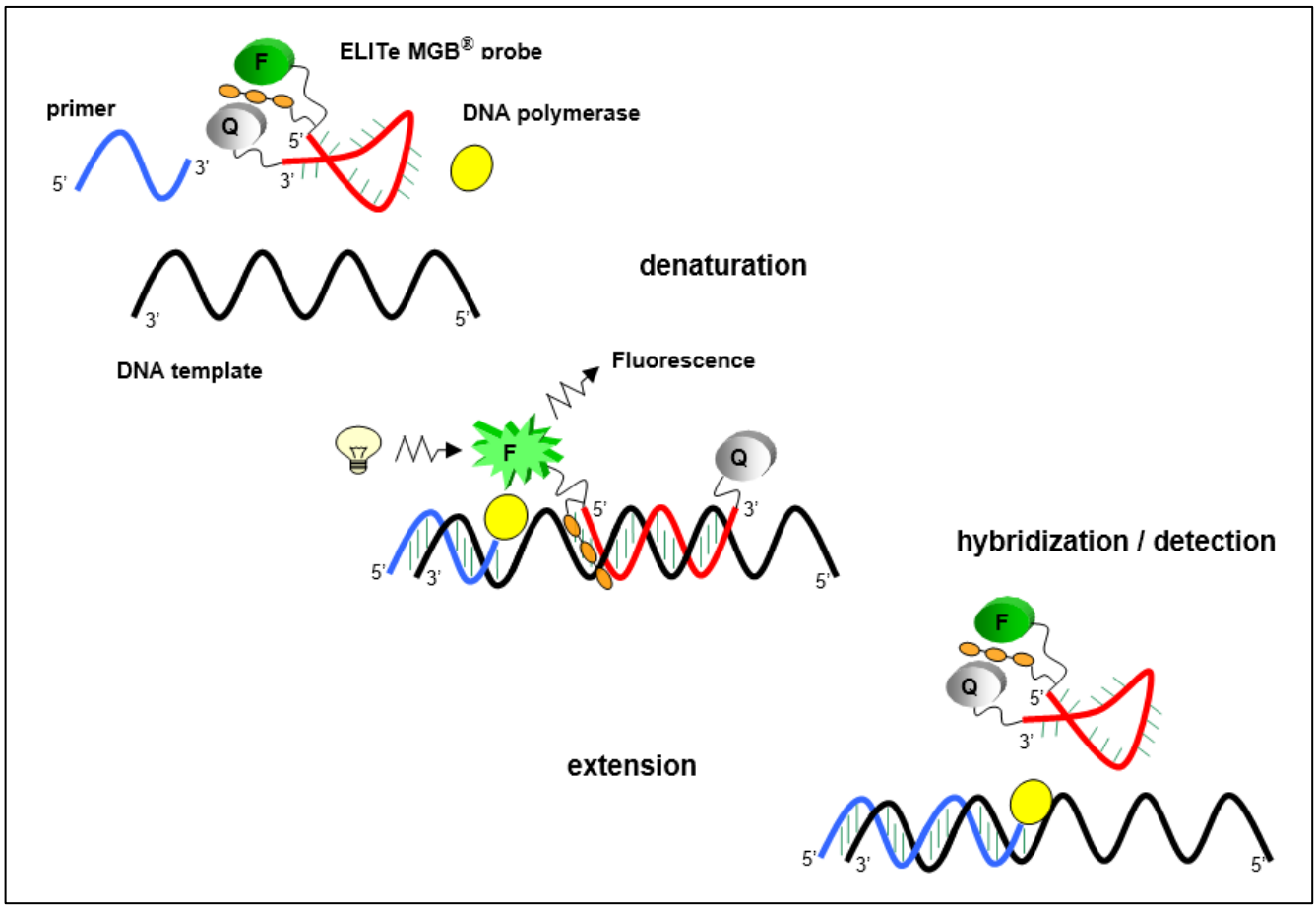
The HSV-1 virus specific probe is manufactured with ELITe MGB technology and labelled with an AP593 fluorophore (ELITech proprietary fluorescent dye with spectral characteristics similar to ROX) and is activated when hybridized with the specific product of the HSV-1 virus amplification reaction.

The HSV-2 virus specific probe is manufactured with ELITe MGB technology and labelled with a FAM fluorophore and is activated when hybridized with the specific product of the HSV-2 virus amplification reaction.

The Internal Control specific probe is manufactured with ELITe MGB technology and labelled with an AP525 fluorophore (ELITech proprietary fluorescent dye with spectral characteristics similar to VIC), and is activated when hybridized with the specific product of Internal Control amplification reaction.

As the specific amplification products in the PCR reaction increase exponentially, the fluorescence emission also increases and is measured and recorded by the instrument. The processing of the data by the ELITe InGenius instrument software determines the presence of HSV-1 and/or HSV-2 virus DNA in the starting sample.

The picture below depicts the mechanism of activation and fluorescence emission of an ELITe MGB probe [3]. Note that the probe is not hydrolyzed during the amplification cycle, which allows it to be utilized for dissociation curve analysis.



MATERIALS PROVIDED IN THE PRODUCT

Box #	Component	Cap Color	Quantity
1	HSV 1&2 Monoreagent	YELLOW	8 × 280 µL
	HSV 1&2 Negative Control	WHITE	2 × 1,800 µL
2	IC2-M13 Internal Control	NEUTRAL	8 × 160 µL
3	HSV 1&2 Positive Control	RED	2 × 1,800 µL
	Sample Dilution Buffer	BROWN	8 × 1,000 µL

PRODUCT DESCRIPTION

The HSV 1&2 ELITe MGB Assay is a multiplexed PCR assay that uses unique primer sets and single uniquely labeled probes to amplify and detect:

- The HSV-1 glycoprotein D encoding gene,
- The HSV-2 glycoprotein G encoding gene, and
- An Internal Control.

Each HSV 1&2 ELITe MGB Assay includes all of the controls and reagents necessary to detect HSV-1 and HSV-2 viruses. Sufficient material is provided to complete 96 reactions at a final volume of 30 µL each.

HSV 1&2 ELITe MGB Assay Components

- **HSV 1&2 ELITe MGB Assay Monoreagent**

  The HSV 1&2 ELITe MGB Assay Monoreagent includes a mixture of primers, probes, dNTPs, buffer, Taq DNA Polymerase, and magnesium chloride. The HSV 1&2 ELITe MGB Assay Monoreagent is provided in 8 tubes, each containing 280 µL of solution.

- **HSV 1&2 ELITe MGB Assay Negative Control**

  The HSV 1&2 ELITe MGB Assay Negative Control is DNase and RNase-free water. The HSV 1&2 ELITe MGB Assay Negative Control is provided in 2 tubes, each containing 1,800 µL of solution.

- **HSV 1&2 ELITe MGB Assay Positive Control**

  The HSV 1&2 ELITe MGB Assay Positive Control is a buffered solution (1X Tris-EDTA, pH 8.0, supplemented with 10mg/mL Yeast RNA) containing the plasmid DNA templates for HSV-1 and HSV-2. HSV 1&2 ELITe MGB Assay Positive Control is provided in 2 tubes, each containing 1,800 µL of solution.

- **IC2-M13 Internal Control**

  The IC2-M13 Internal Control is a buffered solution (LB media) containing recombinant M13 phage with a nonsense, nonspecific internal control DNA fragment cloned into phage genome. The IC2-M13 Internal Control is provided in 8 tubes, each containing 160 µL of solution. The IC2-M13 Internal Control solution is added to all samples prior to extraction to monitor lysis, integrity of the reagents, equipment function, and for the presence of inhibitors.

- **Sample Dilution Buffer**

  The sample dilution buffer is a buffer solution (1X Tris-EDTA, pH 8.0). It is intended to be used for sample dilution when samples being tested are too concentrated, as indicated by the ELITe InGenius software (C_T value < 17, i.e. virus concentration ≥1×10^6 $TCID_{50}$/mL).

MATERIALS REQUIRED BUT NOT PROVIDED

- Laminar airflow hood or Biological Safety Cabinet.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Benchtop microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).

OTHER PRODUCTS REQUIRED

The **ELITe InGenius** instrument, reagents, and consumables **are not** included in this product.

- ELITe InGenius (ELITechGroup MDx LLC, ref. INT030-K)
- ELITe InGenius SP 200 Extraction Cassette (ELITechGroup MDx LLC, ref. INT032SP200)
- ELITe InGenius SP 200 Consumable Set (ELITechGroup MDx LLC, ref. INT032CS)
- ELITe InGenius Waste Box (ELITechGroup MDx LLC, ref. F2102-000)
- ELITe InGenius PCR Cassette (ELITechGroup MDx LLC, ref. INT035PCR)

- Filter tips 300 Axygen (Axygen BioScience Inc., CA, USA, ref. TR-350-LRS)

For performing the **HSV 1&2 ELITe MGB Assay** test on the **ELITe InGenius** instrument (ELITechGroup MDx LLC, ref. INT030-K), the following HSV 1&2 ELITe MGB Assay specific protocols are required:

- Assay Profile: HSV 1&2 ELITe MGB Sample_SW_200_50
- Assay Profile: HSV 1&2 ELITe MGB Positive Control
- Assay Profile: HSV 1&2 ELITe MGB Negative Control
- Control Definition: HSV 1&2 Positive Control
- Control Definition: HSV 1&2 Negative Control
- Reagent: IC2-M13 Internal Control
- Reagent: HSV 1&2 ELITe MGB Monoreagent
- Matrix: Cutaneous and mucocutaneous lesion swab

WARNINGS AND PRECAUTIONS

This product is exclusively designed for *in vitro* diagnostics use.

For IVD use only

General warnings and precautions

1. Carefully read all the instructions provided in the product before running the assay.
2. All procedures in which infectious aerosols or splashes may be created must be conducted in biological safety cabinets.
3. Wear personal protective equipment, such as (but not limited to) gloves and lab coats.
4. Do not eat, drink, smoke or apply cosmetics in the work areas.
5. Do not pipette solutions by mouth.
6. Dispose of unused reagents and specimen waste in compliance with the local, state, and federal regulations.
7. Treat all specimens as if they were able to transmit infective agents.
8. Avoid direct contact with the biological samples.
9. Perform all procedures to minimize the creation of splashes and/or aerosols.
10. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
11. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal.
12. Wash hands after handling samples and before leaving the laboratory.
13. While running the assay, follow the instructions provided in the product.
14. Do not use the product after the indicated expiry date.
15. Only use the reagents provided in the product and those recommended by the manufacturer.
16. Do not use reagents from different lots of materials.
17. Safety Data Sheets are available upon request.

Warnings and precautions for molecular biology

1. Molecular biology procedures, such as nucleic acid extraction, PCR amplification and detection, require qualified and trained staff to avoid the risk of erroneous results.
2. Samples must be handled under a laminar airflow hood or biological safety cabinet.
3. Tubes containing different samples must never be opened at the same time.
4. Pipettes used to handle samples must be exclusively used for this specific purpose.
5. All pipettes must be of the positive displacement type or be used with aerosol filter tips.
6. The tips used must be sterile and free from DNases and RNases.

Warnings and precautions specific for the components

HSV 1&2 ELITe MGB Monoreagent

The HSV 1&2 ELITe MGB Monoreagent must be stored protected from light in a freezer (-20±5°C), and is stable for up to 14 months. The HSV 1&2 ELITe MGB Monoreagent can be frozen and thawed no more than 8 times; further freezing / thawing cycles may cause a loss of product performance. If a spill occurs, the reagent is non-infectious.

Once the tube of HSV 1&2 ELITe MGB Monoreagent is open, placed in and used on ELITe InGenius instrument it **MUST NOT** be re-used and should be discarded.

HSV 1&2 ELITe MGB Assay Negative Control

The HSV 1&2 Negative Control must be stored in a freezer (-20±5°C), and is stable for up to 14 months. If a spill occurs, the reagent is non-infectious.

HSV 1&2 ELITe MGB Assay Positive Control

The HSV 1&2 Positive Control must be stored in a freezer (-20±5°C), and is stable for up to 14 months. Repeated HSV 1&2 Positive Control freeze/thaw cycles should be avoided (no more than 8 times) and may cause a loss of product performance. If a spill occurs, the reagent is non-infectious.

IC2-M13 Internal Control

The DNA Internal Control must be stored in a freezer (-20±5°C), and is stable for up to 14 months. Repeated DNA Internal Control freeze/thaw cycles should be avoided (no more than 8 times) and may cause a loss of product performance. If a spill occurs, the reagent is non-infectious.

Once a tube of IC2-M13 Internal Control is opened, placed in, and used on ELITe InGenius instrument it **MUST NOT** be re-used and should be discarded.

Sample Dilution Buffer

The sample dilution buffer must be stored in a freezer (-20±5°C), and is stable for up to 14 months. If a spill occurs, the reagent is non-infectious.

ELITe InGenius Training

The HSV 1&2 ELITe MGB Assay is intended for use by trained clinical laboratory personnel who have received specific training on the use of the HSV 1&2 ELITe MGB Assay on the ELITe InGenius instrument. The training for use of the HSV 1&2 ELITe MGB Assay and ELITe InGenius instrument will be provided by ELITechGroup technical services.

INSTRUCTION FOR USE

Running the HSV 1&2 ELITe MGB Assay consists of three steps:

- System readiness verification
- Set up of the session
- Review and approval of results

System Readiness Verification

Before starting the session, it is necessary to:

- Switch on ELITe InGenius and select the mode “CLOSED”;
- Verify that the amplification Controls (HSV 1&2 ELITe MGB Positive Control, HSV 1&2 ELITe MGB Negative Control) have been run, approved and are not expired (status). This can be checked under the “Control” menu in the Home page;
- Choose the type of run and set up the run, following the instructions for the session set up and using the Assay Protocols identified below. These protocols were specifically validated with ELITe MGB Assays and ELITe InGenius.

The Assay protocols for the HSV 1&2 ELITe MGB Assay are:

- Assay Profile: HSV 1&2 ELITe MGB Sample_SW_200_50

- Assay Profile: HSV 1&2 ELITe MGB Positive Control
- Assay Profile: HSV 1&2 ELITe MGB Negative Control
- Control Definition: HSV 1&2 Positive Control
- Control Definition: HSV 1&2 Negative Control
- Reagent: IC2-M13 Internal Control
- Reagent: HSV 1&2 ELITe MGB Monoreagent
- Matrix: Swab

If the HSV 1&2 ELITe MGB Assay protocols required to perform the assay are not installed on the ELITe InGenius system, contact your local ELITechGroup Customer Service (see contact information on page 1).

Setup of the PCR Session

The HSV 1&2 ELITe MGB Assay in association with ELITe InGenius, can be used to perform an integrated run with one Positive Control, one negative control, and samples (Extract + PCR).

The HSV 1&2 ELITe MGB Assay amplification thermal profile is included in the protocol available for the ELITe InGenius instrument, and is automatically loaded when the assay protocol is selected.

The main steps for the setup of a run are described below.

Integrated run (Extract + PCR) with Positive and Negative Controls.

To set up the integrated run (Extract + PCR) with one Positive Control, one Negative Control, and test samples to be analyzed, carry out the steps below. The HSV 1&2 ELITe MGB Positive Control and HSV 1&2 ELITe MGB Negative Control should be included with the first run each day, on each instrument, but can be omitted in subsequent runs.

1. Thaw the primary sample tubes to be analyzed (if frozen), mix by vortexing, briefly spin down each tube (500×g for 10 seconds) to bring the content to the bottom, and then transfer the samples to the ELITe InGenius instrument. Keep samples in a refrigerator or on ice if planning to use later the same day (within 8 hours).
2. Remove the tube of the HSV 1&2 ELITe MGB Positive Control (RED cap) and warm to room temperature. The tube is sufficient for 8 Extract+PCR runs. Mix by vortexing and briefly spin down the tube (500×g for 10 seconds) to bring the content to the bottom.
3. Remove the tube of the HSV 1&2 ELITe MGB Negative Control (WHITE cap) and warm to room temperature. The tube is sufficient for 8 sessions. Mix by vortexing, briefly spin down the tube (500×g for 10 seconds) to bring the content to the bottom.
4. Thaw a tube of the HSV 1&2 ELITe MGB Monoreagent (YELLOW cap), mix by vortexing, briefly spin down the tube (500×g for 10 seconds) to bring the content to the bottom, and then keep in a refrigerator or on ice until ready to transfer to the ELITe InGenius instrument. The tube is sufficient for 1 session (12 PCR reactions).

   Note: Once the tube of HSV 1&2 ELITe MGB Monoreagent is open, placed in and used on ELITe InGenius instrument it **MUST NOT** be re-used and should be discarded.
5. Thaw a tube of the IC2-M13 Internal Control DNA (NEUTRAL cap), mix by vortexing, briefly spin down the tube (500×g for 10 seconds) to bring the content to the bottom, and then keep in a refrigerator or on ice until ready to transfer to use. The tube is sufficient for 1 session (12 PCR reactions).

   Note: Once the tube of IC2-M13 Internal Control is open, placed in and used on ELITe InGenius instrument it **MUST NOT** be re-used and should be discarded.
6. Using the ELITe InGenius Graphical User Interface (GUI), select “Perform Run” from the Home screen, for each track to be loaded.
   a. Choose “Extracted Elute Volume” of 50 µL from the Instrument menu.
   b. In the “Assay” column select the assay protocol for the Positive Control, Negative Control, and each sample:
      i. In the “Assay” column, select the “HSV 1&2 ELITe MGB Positive Control” protocol for the ELITe InGenius track being prepared for the HSV 1&2 ELITe MGB Positive Control. Fill in the lot number and expiry date, and then click on “Next” to continue the setup.

         Note: This step is optional if the Positive Control has already been run that day on the ELITe

InGenius instrument being used. The control should be tested following the frequency required by state and federal regulations

ii. In the “Assay” column, select the “HSV 1&2 ELITe MGB Negative Control” protocol for the ELITe InGenius track being prepared for the HSV 1&2 ELITe MGB Negative Control. Fill in the lot number and expiry date, and then click on “Next” to continue the setup.

Note: This step is optional if the Negative Control has already been run that day on the ELITe InGenius instrument being used. The control should be tested following the frequency required by state and federal regulations

iii. In the “Assay” column, select the “HSV 1&2 ELITe MGB Sample_SW_200_50” protocol for each ELITe InGenius track being prepared for a sample.

c. In the “SampleID” (SID) column, manually enter the sample name or scan the sample barcode for each ELITe InGenius track being prepared for a sample.

Note: The SampleID, Sample matrix, Protocol and Sample position columns for the Positive Control and Negative Control are added automatically during the control setup and cannot be modified.

d. In the “Protocol” column, check that “Extract + PCR” is displayed for each ELITe InGenius track being prepared for a sample.

e. In the “Sample Position” column, select “Primary tube“ if using the primary tube compatible with InGenius and a sample volume > 2.2 mL. Select “Sonicator Tube” for the sample loading position if the sample volume is < 2.2 mL or contained in a primary tube not compatible with InGenius.

f. Click “Next” to continue the setup.

7. Select a Reagent Cold Block (Inventory Manager) for the HSV 1&2 ELITe MGB Monoreagent and IC2-M13 Internal Control DNA.
   a. Select a position for the HSV 1&2 ELITe MGB Monoreagent and manually enter the lot number, expiration date, and number of tests prepared.
   b. Following the GUI load the HSV 1&2 ELITe MGB Monoreagent into the Reagent Cold Block selected position and remove the cap.
   c. Select a position within the Reagent Cold Block for the IC2-M13 Internal Control DNA and manually enter the lot number, expiration date, and number of tests prepared.
   d. Following the GUI load the IC2-M13 Internal Control DNA into the Reagent Cold Block selected position and remove the cap.

   Note: Refer to the SCH mINT030-K_En ELITe InGenius System Operator’s Manual to setup the Reagent Cold Block (Inventory Manager) if necessary.
8. Load the ELITe InGenius Inventory Area with reagents, tips, and a waste box:
   a. Check that the individual pipettor tip racks in the Inventory Area contain the required number of tips following the GUI.
   b. Load an ELITe InGenius “Waste Box” following the GUI.
9. Load the ELITe InGenius instrument with cassettes for each track:
   a. Load the required number of “ELITe InGenius SP 200” extraction cassettes following the GUI.
   b. Load the required number of “PCR Cassette” consumables following the GUI.
10. Load the ELITe InGenius instrument “ELITe InGenius SP 200 Consumable Set” tip cartridges and tubes for each track following the GUI.
    a. Load the required number of uncapped 0.5 mL Sarstedt tubes into the front row of the eluate collection rack following the GUI.
    b. Remove the metal grid cover from the tip cartridge loading position, load the required number of tip cartridges, and then securely replace the metal grid cover following the GUI.
    c. Load the required number of sonicator tubes into the sonication tube rack following the GUI, and then place into the InGenius. If using sonicator tubes for sample of small volume, set aside the loaded sonicator rack for sample loading.
11. Control loading
    a. Carefully transfer 0.2 mL of HSV 1&2 ELITe MGB Positive Control and HSV 1&2 ELITe MGB

Negative Control into the corresponding sonicator tubes in the prepared sonication rack in a laminar flow hood or biological safety cabinet. Follow the positions selected above in step 6 for correct placement of each control in the sonicator rack.

12. Sample Loading
    a. If the samples tubes **are** compatible (polypropylene 12 x 80mm screw cap tube with internal conical shape, Copan Diagnostics, or similar) with the InGenius primary tube sample rack, and the sample volume is > 2.2 mL, load the samples into the rack and transfer it into the ELITe InGenius instrument into pre-selected (step 6) positions.
    b. If the samples are **not** compatible with the InGenius primary tube sample rack **or** if the volume of the sample is ≤ 2.2 mL, carefully transfer 0.2 mL of each sample into the sonicator tubes in the prepared sonicator rack in a laminar flow hood or biological safety cabinet. Follow the positions selected above in step 6 for correct placement of each sample in the sonicator rack.

    Note: The sonicator tubes are placed into the sonication rack and are open during the loading process. The sonication rack separates the tubes by 1 cm from tube to tube with a metal splash guard between each tube position.

    c. Load the primary tube sample rack (if the samples tubes **are** compatible with the ELITe InGenius instrument) and sonicator rack containing the HSV 1&2 ELITe MGB Positive Control, HSV 1&2 ELITe MGB Negative Control and samples (if the samples tubes **are not** compatible with the ELITe InGenius instrument) into the ELITe InGenius instrument.

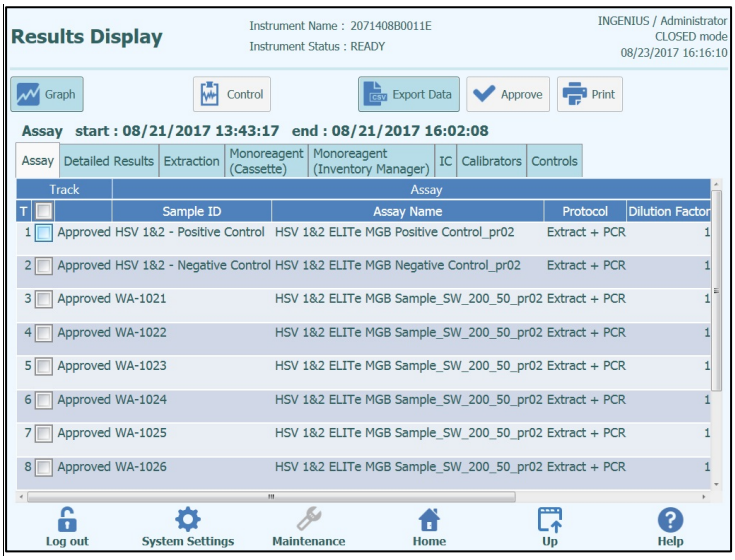
    Note: Check that all of the consumables, cassettes, tubes, and racks are properly loaded and are ready for the run to begin.
13. Close the instrument door.
14. Press “Next” after each step when prompted by the ELITe InGenius GUI to confirm that the consumables, cassettes, tubes, and racks are loaded in the correct positions. The final step is to press “Start” to begin the run.
15. Verify the instrument door is closed. Re-adjust the door if the corresponding message appears.
16. After the run is completed, the “Results Display” screen is automatically displayed starting on the “Assay” tab. This window allows the user to view, approve, or store the results and to print and save a report.

    Note: At the end of the run all cassettes, consumables, and samples must be removed from the instrument and disposed in accordance with local, state, and federal regulations.

Review and approval of results:

At the end of the run, the “Results Display” screen is automatically displayed starting on the “Assay” tab. The example in the figure below shows the sample and control results and additional information regarding the run.

Results Display



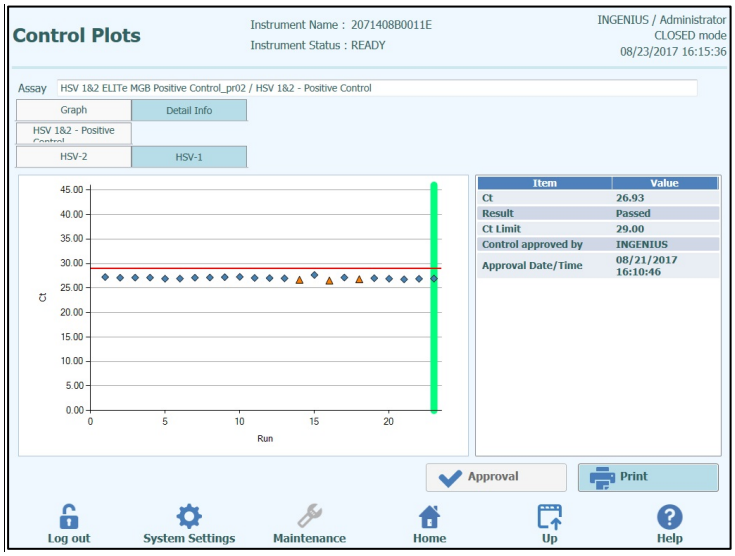
The ELITe InGenius software requires approval of the positive and Negative Controls before any sample results can be approved. The positive and Negative Control results are specific for the reagent lot and will expire daily.

Approval of results is a two-step process.

1. Determine if the control & sample results are valid.

   From the “Results Display” screen, check the box on the left side of the screen for the track containing the “HSV 1&2 ELITe MGB Positive Control”, and then click on the “Control” button to initiate the approval dialog window. For additional details regarding the approval process, see the ELITe InGenius System Operator’s Manual. The example in the figure below shows the HSV 1&2 ELITe MGB Positive Control approval screen. If any results are invalid the run (in case of an invalid control) and sample will require retesting.

Control Plots: Positive Control Approval



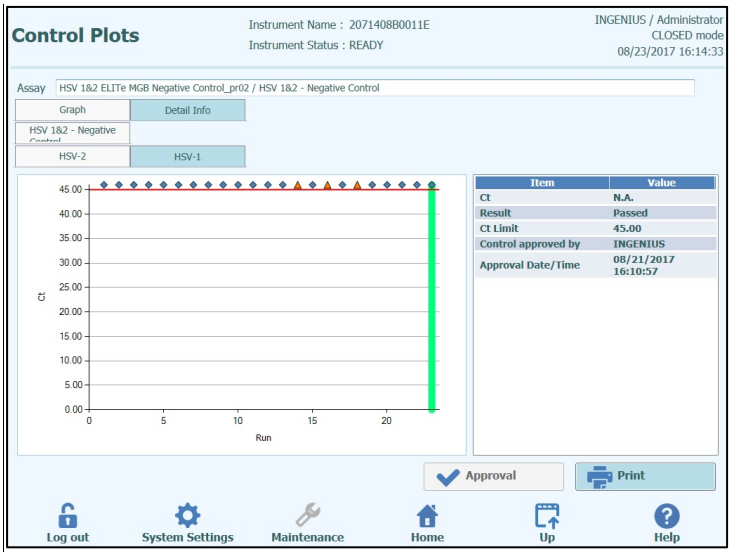
Note: When the HSV 1&2 ELITe MGB Positive Control result does not meet the acceptance criteria, the “not passed” message is shown on the “Controls” menu and it is not possible to approve the result. The HSV 1&2 ELITe MGB Positive Control reaction has to be repeated.

Note: When the HSV 1&2 ELITe MGB Positive Control is run together with samples and its result is invalid, the entire run is invalid and all samples must be repeated.

From the “Results Display” screen, check the box on the left side of the screen for the track containing the “HSV 1&2 ELITe MGB Negative Control”, and then click on the “Control” button to initiate the

approval dialog window. For additional details regarding the approval process, see the ELITe InGenius System Operator's Manual. The example in the figure below shows the HSV 1&2 ELITe MGB Negative Control approval screen.

Control Plots: Negative Control Approval



Before analyzing any sample, it is mandatory to generate and to approve a Negative Control result for each run containing a Negative Control.

Note: When the HSV 1&2 ELITe MGB Negative Control result does not meet the acceptance criteria, the "not passed" message is shown on the "Controls" menu and it is not possible to approve the result. The HSV 1&2 ELITe MGB Negative Control reaction has to be repeated.

Note: When the HSV 1&2 ELITe MGB Negative Control is run together with samples and its result is invalid the entire session is invalid and all samples must be repeated.

a. Validation of HSV 1&2 ELITe MGB Sample Result Approval

The fluorescence signals emitted by the specific HSV 1&2 ELITe MGB Assay probes in the HSV 1&2 ELITe MGB sample reaction are analyzed automatically at the end of the run by the ELITe InGenius software.

On the "Results Display" screen, check the box on the left side of the screen for each track containing a test sample, and then click on the "Approve" button

Note: It is mandatory to approve daily the HSV 1&2 ELITe MGB Positive Control and HSV 1&2 ELITe MGB Negative Control results for the current lot of reagent before any sample results can be approved. To check the status of the controls, the status is shown in the "Calibration" and "Controls" windows of ELITe InGenius software.

The Sample run results are stored in the database and, if valid, can be approved (Result Display) by "Administrator" or "Analyst" personnel using the ELITe InGenius software.

Results are described in the reports generated by the instrument ("Result Display").

Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and other laboratory test outcomes concerning the patient.

2. Sample Result Reporting

After the assay results have been approved, assay result reports can be printed and saved. The sample results are stored in the database and can be exported as "Sample Report" and "Track Report".

i. The "Sample Report" shows the details of a sample run sorted by Sample ID (SID).

ii. The "Track Report" shows the details of a sample run track by selected track.

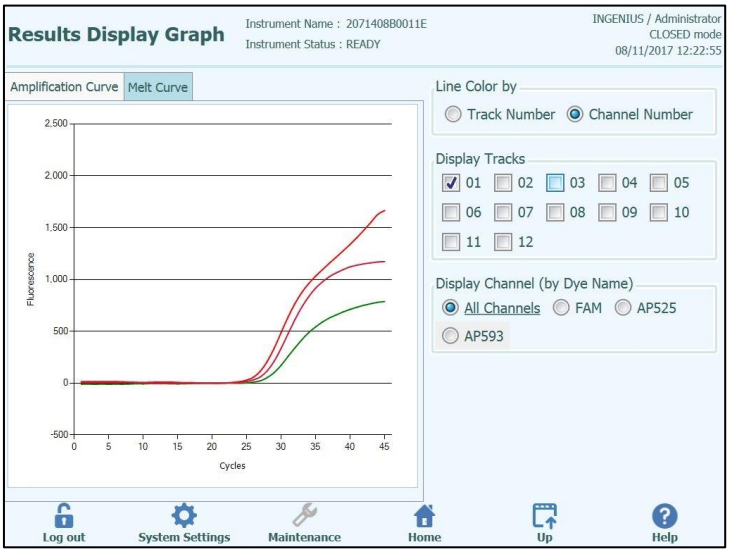
The "Sample Report" and "Track Report" can be printed or saved as a PDF document for printing later. The report should be reviewed and then signed by authorized personnel to keep as a record.

a. Results Display

From the “Results Display” screen, select the “Graph” button.

This will display the amplification plot for each selected sample. The example in the figure below shows the Results Display Graph (amplification curve) screen.

Results Display Graph: Amplification Curve

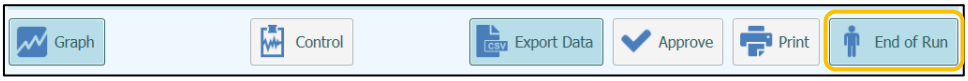


The selected samples can be viewed by track number or channel number. The samples can also be displayed in all channels (default) or a single channel can be selected. Each individual sample can also be individually displayed by either selecting (all samples selected by default) or deselecting a sample.

b. Export Data: From the “Results Display” screen, select the “Export Data” button to export the run data.

c. Print: From the “Results Display” screen, select the “Print” button to generate a report of selected tracks, and then print the report or save it as a PDF document for printing later. Verify the controls are valid, and the data is reported correctly, then sign the document to keep as a record.

d. End of Run: After the run is completed and the results have been approved and reported, press the “End of Run” button. This will unlock the ELITe InGenius instrument and allow the user to dispose of all of the consumables and reagents.



Note: The cassettes, consumables, and samples must be removed from the instrument and disposed in accordance with local, state, and federal regulations.

Note: For detailed information refer to ELITe InGenius System Operator’s Manual.

RESULTS AND INTERPRETATION

Table 1. Summary of the Run Validity Criteria and Result Interpretation Algorithm:

HSV-1 C_T value AP593, Channel 4	HSV-2 C_T value FAM, Channel 1	IC C_T value AP525, Channel 2	Status	HSV-1 Result	HSV-2 Result
Undetermined $C_T \geq 45.0$	Undetermined $C_T > 45.0$	$C_T \leq 32.0$	Valid	Negative	Negative
		Undetermined or $C_T > 32.0$	Invalid	Invalid	Invalid
Determined $C_T \leq 45.0$	Undetermined $C_T > 45.0$	NA	Valid	Positive	Negative
	Determined $C_T \leq 45.0$	NA		Positive	Positive
Undetermined $C_T > 45.0$	Determined $C_T \leq 45.0$	NA	Valid	Negative	Positive

The Sample run is valid when the conditions reported in the table below are met.

1) HSV 1&2 ELITe MGB Positive Control	Status
HSV 1&2 ELITe MGB Positive Control	APPROVED
2) HSV 1&2 ELITe MGB Negative Control	**Status**
HSV 1&2 ELITe MGB Negative Control	APPROVED

ELITe InGenius software reports as target DNA being “Detected” or “Not Detected”. For a valid run, the specimen results are interpreted as follows.

Table 2. ELITe InGenius Software Output and Interpretation

Results of Sample Run		Interpretation
HSV-1 Result	HSV-2 Result	
HSV-1 DNA Not Detected	HSV-2 DNA Not Detected	HSV-1 and HSV-2 DNA not detected
HSV-1 DNA Not Detected	HSV-2 DNA Detected	HSV-1 DNA not detected and HSV-2 DNA detected
HSV-1 DNA Detected	HSV-2 DNA Not Detected	HSV-1 DNA detected and HSV-2 DNA not detected
HSV-1 DNA Detected	HSV-2 DNA Detected	HSV-1 and HSV-2 DNA detected
Invalid - Retest Sample		Invalid assay result due to Internal Control failure (Incorrect extraction or presence of inhibitor). Specimen Should be retested

Samples not suitable for analysis are reported as “Invalid” by the ELITe InGenius software Follow up the “Invalid” results as described below:

a. The results are negative for HSV-1, HSV-2 and also for the internal control.

   The following error message will appear: “Invalid - Retest Sample”. Retest the sample as described in section 12 of this manual.

b. The sample has a C_T value <17, virus concertation $\geq 1 \times 10^6$ $TCID_{50}$/mL.

   The following error message will appear: “Invalid-Failed to calculate Ct”. The results are positive for either HSV-1 or HSV-2. To obtain Cts dilute the sample by pipetting 10 µL of the sample and adding it directly to the sample dilution buffer tube (brown capped tube) supplied in the Assay. Cap the tube, vortex, briefly spin down and test the diluted sample as described in section 12b of this manual.

Samples suitable for analysis in which HSV-1 and/or HSV-2 virus DNA is not detected are reported as: “HSV-1 DNA not detected” and/or “HSV-2 virus DNA not detected".

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The HSV 1&2 ELITe MGB Assay product must be used in conjunction with the ELITe InGenius instrument to perform the extraction of DNA from cutaneous and mucocutaneous lesion swab specimens collected in a clinical setting. The clinical samples must be collected with swabs and stored in UTM, M4, M4RT, M5 or M6 viral transport media according to laboratory guidelines and stored for a maximum of 7 days in a refrigerator (4±3°C) or at -70°C for a maximum of 4 month.

The specimens should be transported following the local and national instructions for the transport of pathogenic material.

SPECIMEN PROCESSING

Note: Use the extraction protocol "HSV 1&2 ELITe MGB Sample_SW_200_50" to perform DNA extraction from swab specimens on the ELITe InGenius instrument with the ELITe InGenius Software version 1.2 (or later equivalent versions). This protocol processes 200 µL of sample, adds the Internal Control at 10 µL/extraction and elutes the nucleic acids in 50 µL of Sample Elution Buffer.

Samples provided in an ELITe InGenius compatible primary tube (polypropylene 12×80mm screw cap tube with internal conical shape, Copan Diagnostics, or similar) with a sample volume of > 2.2 mL can be placed directly in the ELITe InGenius primary sample rack. Samples provided in a tube that is not compatible with the ELITe InGenius or that has a sample volume < 2.2 mL require a 200 µL aliquot to be transferred into a prepared sonicator tube, and placement in the ELITe InGenius sonicator tube rack. Refer to the ELITe InGenius Operator's Manual (SCH mINT030-K_en) for more information.

QUALITY CONTROLS

Daily validation and approval of a Positive and Negative Controls provided with the assay is required on the first amplification session.

Negative Control: use the provided Negative Control in place of a sample.

Positive Control: use the provided HSV 1&2 ELITe MGB Positive Control in place of a sample.

Nucleic acid extraction control: use the provided DNA Internal Control, 10 µL of which will be automatically added from the tube in the Reagent Cold Block to each sample or control during the extraction step.

Note: ELITe InGenius with ELITe InGenius Software allows for validation of amplification controls for each lot of amplification reagent to be approved and stored in its database. Amplification validation control results will expire each day and it will be necessary to re-run the Positive and Negative Controls.

The amplification controls must be retested if any of the following events occurs:

- A new lot of amplification reagents is started
- The results of Positive Control or Negative Control analysis are out of specification
- Any major maintenance is performed on ELITe InGenius

Note: Control ranges may vary. Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

MAINTENANCE

Maintenance by ELITechGroup

Periodic maintenance by ELITechGroup is necessary for the ELITe InGenius system to ensure appropriate performance. ELITechGroup service staff will provide appropriate maintenance and inspection on an annual basis for the duration of the service contract.

Maintenance by User

Regular Maintenance

In case of spills of reagent or samples, wipe the spill with a dry lint-free cloth and then wash and clean by using lint-free tissue moistened with 70% ethanol. Treat all used cleaning material as potentially infectious.

Also at the end of each run:

- Remove and discard any PCR Cassettes from the PCR Rack.
- Remove and discard any extraction cassettes from the extraction rack.
- Remove and discard any extraction Tip Packages.
- Remove and discard any sonicator tubes from the Sonication Rack.
- Remove and discard the remainder of Monoreagent and IC2-M13 from cooling block.
- Fit caps to the Elution tubes and remove the tubes for storage (if necessary). Otherwise dispose them.
- Re-seal caps to the Primary sample tubes (if any). Store as recommended.

Daily Maintenance

Perform daily maintenance at the end of each day, after regular maintenance.

- Empty and replace the waste box liner at the end of each day.
- Remove all removable objects (tube carriers, adapters, racks, inserts, tip racks) from the instrument. **Note:** Do not refill used tip racks.
- Using a lint free cloth dampened with 70% ethanol wipe all interior surfaces of the instrument. Dispose of used cleaning material as potentially infectious.
- UV Decontamination.

It is recommended to perform a UV decontamination each day that the instrument is used.

Weekly Maintenance

Note: Turn off the instrument when it has been cleaned.

- Remove all removable objects (tube carriers, adapters, racks, inserts) from the instrument.
- Clean the removed objects by soaking them in a quaternary ammonium salt-based disinfectant (e.g. DECON-QUAT) according to the manufacturer‘s instructions for at least 15 min.

  DECON-QUAT - quaternary ammonium salt based disinfectant concentrate (contains 5% alkyldimethylbenzylammonium chloride and 5% alkyldimethylethylbenzylammonium chloride). 1×solution is used to remove nucleic acid contamination (DNA and RNA).
- After incubation, rinse the removed objects thoroughly with water and wipe dry with lint-free tissues.

To clean the hoods of the ELITe InGenius instruments, wipe the surface with a soft lint-free cloth moistened with deionized water. Then wipe dry with a dry soft lint-free paper towel.
IMPORTANT: Do not use ethanol-based disinfectant; use distilled water instead.

LIMITATIONS

HSV-1 Detection was found to be inhibited in the presence of HSV-2 DNA titers of 1×10^3 $TCID_{50}$/mL or higher in the analytical interference study

Only for use with the following clinical samples: patients’ lesion swab samples stored in UTM and M4, M4RT, M5 or M6 medium.

The device performance was not evaluated in urethral, ocular and nasal mucocutaneous lesions samples.

The results obtained with this product depend on following the instructions for use.

False-negative results and false-positive results can arise

This device must be handled by qualified personnel trained on the ELITe InGenius instrument in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

Possible polymorphisms within the region of the viral genome covered by the product primers and probes may impair detection of HSV-1 and/or HSV-2 virus DNA.

The results obtained with this product must be interpreted taking into consideration all the clinical findings and other laboratory tests done on the patient.

Negative results do not preclude HSV-1 and/or HSV-2 virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations and patient history.

PERFORMANCE CHARACTERISTICS

A. Clinical Evaluation

a. Study Description

To evaluate the clinical performance of the HSV 1&2 ELITe MGB Assay, device performance was compared to a composite reference method. It consisted of an FDA cleared assay and a validated HSV 1&2 PCR followed by bi-directional sequencing of gel electrophoresis-positive samples). Validated HSV 1&2 PCR targeted genomic regions distinct from the HSV 1&2 ELITe MGB Assay. A positive result by the composite reference method is defined as a positive by the FDA cleared PCR or the validated sequencing. Two negative results are needed to confirm a negative)

A total of 1,174 left-over prospectively collected archived swab samples from cutaneous (546) and mucocutaneous (628) lesions from symptomatic patients were collected and evaluated in the study.

The samples were tested with HSV 1&2 ELITe MGB Assay and the Composite Reference Method. Out of the 1,174 tested samples 2 samples were found invalid by the ELITe MGB Assay and were excluded from the performance analysis tables.

Out of the 1172 remaining samples 1 additional invalid sample result for HSV-1 and 2 additional invalid sample results for HSV-2 by the composite reference method were removed from the performance analysis tables.

Therefore, for HSV-1, 1171 samples analyzed and for HSV-2 1170 samples were analyzed.

b. Results: Expected Values/Reference Range

HSV 1&2 Prevalence

The observed expected values for HSV-1 and HSV-2 in the study population using the HSV 1&2 ELITe MGB Assay were calculated for cutaneous and mucocutaneous specimens and is summarized for the combined sample set per age group, by gender and by specimen source in the tables below. A total number of 6 dual positives for HSV-1 and HSV-2 detected by the ELITe MGB Assay and one of the samples was confirmed positive by the composite reference method.

Table 3: Cutaneous and Mucocutaneous HSV 1&2 Prevalence by Age and Gender

Gender	Age Group	Total	HSV 1&2 ELITe MGB Assay HSV-1 results		HSV 1&2 ELITe MGB Assay HSV-2 results	
			Positive	Prevalence	Positive	Prevalence
Female	<20	42	18	42.9%	12	28.6%
	20-29	244	68	27.9%	70	28.7%
	30-39	143	24	16.8%	45	31.5%
	40-49	97	14	14.4%	25	25.8%
	50-59	88	18	20.5%	24	27.3%
	≥60	123	21	17.1%	30	24.4%
	All	737	163	22.1%	206	28.0%
Male	<20	20	4	20.0%	2	10.0%
	20-29	144	25	17.4%	33	22.9%
	30-39	117	15	12.8%	25	21.4%
	40-49	48	5	10.4%	15	31.3%
	50-59	44	6	13.6%	9	20.5%
	≥60	61	5	8.2%	13	21.3%
	All	434	60	13.8%	97	22.4%
	Gender is not identified	1	0	0%	0	0%
ALL		1172	223	19.0%	303	25.9%

Table 4: Cutaneous HSV 1&2 Prevalence by Lesion Source

Lesion Source	Total	HSV 1&2 ELITe MGB Assay HSV-1 results		HSV 1&2 ELITe MGB Assay HSV-2 results	
		Positive	Prevalence	Positive	Prevalence
Genital/Anogenital	248	38	15.3%	78	31.5%
Skin lesion	297	47	15.8%	53	17.8%
All	**545**	**85**	**15.6%**	**131**	**24.0%**

Table 5: Mucocutaneous HSV 1&2 Prevalence by Lesion Source

Lesion Source	Total	HSV 1&2 ELITe MGB Assay HSV-1 results		HSV 1&2 ELITe MGB Assay HSV-2 results	
		Positive	Prevalence	Positive	Prevalence
Genital/Vaginal/Cervical	501	109	21.8%	163	32.5%
Oral	74	21	28.4%	2	2.7%
Other	27	5	18.5%	2	7.4 %
Anorectal	12	2	16.7%	5	41.7%
Urethral	6	0	0 %	0	0 %
Ocular	5	0	0 %	0	0 %
Nasal	2	1	50.0 %	0	0 %
All	**627**	**138**	**22.0%**	**172**	**27.4%**

c. Results: Clinical Performance

HSV-1 Positive/Negative Percent Agreements (PPA/NPA) - Summary of the Results:

The PPA/NPA performance of HSV 1&2 ELITe MGB Assay when compared to the Composite Reference Method in detection of HSV-1 DNA in cutaneous and mucocutaneous lesions is summarized in the table below:

Table 6: Summary of HSV-1 Results for Valid Cutaneous Lesion Samples (N=545)

HSV 1&2 ELITe MGB Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	78	7	85
Negative	1	459	460
Total	79	466	545
		95% CI	
PPA	98.7% (78/79)	93.2-99.8%	
NPA	98.5% (459/466)	96.9-99.3%	

Table 7: Summary of HSV-1 Results for Valid Mucocutaneous Lesion Samples (N=626)

HSV 1&2 ELITe MGB Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	126	12	138
Negative	1	487	488
Total	127	499	626
		95% CI	
PPA	99.2% (126/127)	95.7-99.9%	
NPA	97.6% (487/499)	95.8-98.6%	

HSV-2 PPA/NPA- Summary of the Results:

The PPA/NPA performance of HSV 1&2 ELITe MGB Assay when compared to the Composite Reference Method in detection of HSV-2 DNA in cutaneous and mucocutaneous lesions is summarized in the table below:

Table 8: Summary of HSV-2 Results Valid Cutaneous Lesion Samples (N=545)

HSV 1&2 ELITe MGB Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	125	6	131
Negative	5	409	414
Total	130	415	545
		95% CI	
PPA	96.2% (125/130)	91.3-98.3%	
NPA	98.6% (409/415)	96.9-99.3%	

Table 9: Summary of HSV-2 Results for Valid Mucocutaneous Lesion Samples (N=625)

HSV 1&2 ELITe MGB Assay	Composite Reference Method		
	Positive	Negative	Total
Positive	164	8	172
Negative	4	449	453
Total	168	457	625
		95% CI	
PPA	97.6% (164/168)	94.0-99.1%	
NPA	98.2% (449/457)	96.6-99.1%	

d. HSV-2 Contrived Oral Panel Study

Due to the difficulty in obtaining sufficient HSV-2 positive oral samples, testing for HSV-2 was supplemented by using a contrived panel. The panel consisted of 75 individual negative cheek swab samples collected in Universal Transport Media (UTM) and spiked with HSV-1 and HSV-2 at various concentrations (as shown in table below).

Table 10: HSV Oral Contrived Sample Panel

Level	Sample #	Contrived sample titer	× LoD
Level 1	10	HSV-2 Positive @ 5,400 $TCID_{50}$/mL	1,000×LoD
Level 2	10	HSV-2 Positive @ 1,080 $TCID_{50}$/mL	200×LoD
Level 3	10	HSV-2 Positive @ 216 $TCID_{50}$/mL	40×LoD
Level 4	10	HSV-2 Positive @ 43.2 $TCID_{50}$/mL	8×LoD
Level 5	10	HSV-2 Positive @ 16.2 $TCID_{50}$/mL	3×LoD
Level 6	10	HSV-1 Positive @ 590 $TCID_{50}$/mL	10×LoD
Level 7	15	HSV-1/HSV-2 Negative Oral Samples	
	75	**Samples Total**	

All panel members were randomized, blinded to the tester and tested with HSV 1&2 ELITe MGB Assay on the ELITe InGenius instrument according to the clinical study protocol.

The HSV-2 Oral Contrived Panel Study revealed that 49 out of 50 oral HSV-2 contrived samples were positive using HSV 1&2 ELITe MGB Assay (98% detection). All 10 HSV-1 Positive samples confirmed 100% positivity.

e. Reproducibility

The reproducibility of the HSV 1&2 ELITe MGB Assay was evaluated in a multi-site investigation using contrived clinical samples. HSV test panels were prepared by spiking HSV-1 (MacIntyre strain) or HSV-2 (MS strain) virus into UTM (Universal Transport Media) at the concentrations of <1× LoD, 1 × LoD and 3 × LoD. HSV-1 and HSV-2 negative panel members were included as panel member controls. The reproducibility panel composition is shown in the table below:

Table 11: Reproducibility Panel

Name	Description of Contents	Viral Load	Expected Positivity Rate
M1	HSV-1 C_{50} (High Negative) in UTM	<1× LOD	20-80% positive
M2	HSV-1 C_{95} (Low Positive) in UTM	1× LOD	≥95% positive
M3	HSV-1 C_{100} (Moderate Positive) in UTM	2-3 × LOD	100% positive

M4	HSV-2 C_{50} (High Negative) in UTM	<1× LOD	20-80% positive
M5	HSV-2 C_{95} (Low Positive) in UTM	1× LOD	≥95% positive
M6	HSV-2 C_{100} (Moderate Positive) in UTM	2-3 × LOD	100% positive
M7	HSV Negative in UTM	Negative	100% negative

Panels were tested at 3 sites by 2 operators per site with 1 run per operator per day, for 10 non-consecutive days using a single lot of HSV 1&2 ELITe MGB Assay. Testing was performed on a minimum of 90 (30 per site) replicates per panel member. Lot-to-Lot variability was assessed only at EGI MDx (internal site) using 3 lots of HSV 1&2 ELITe MGB Assay. Controls were run daily and were included in the first run of the day.

% Agreement, average Cts and %CV for each panel member and per each site are presented in the table below.

Table 12: HSV 1&2 ELITe MGB Assay Reproducibility Results

		Site – 1			Site – 2			Site – 3				
Target	**Sample**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**95% CI**
HSV-1 Result	HSV-1 Low Pos	100.0% (30/30)	38.9	1.70%	100.0% (30/30)	38.3	2.10%	100.0% (30/30)	38	2.00%	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Mod Pos	100.0% (30/30)	36.4	1.30%	100.0% (30/30)	35.5	5.20%	100.0% (30/30)	35.6	1.50%	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Pos	100.0% (30/30) [a]	NA	NA	100.0% (29/29) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (89/89)	95.6 to 100.0%
	HSV-2 Mod Pos	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (30/30) [a]	NA	NA	100.0% (90/90)	95.9 to 100.0%
	HSV Neg	100.0% (60/60) [a]	NA	NA	100.0% (38/38) [a]	41.4	2.50%	100.0% (40/40) [a]	NA	NA	100.0% (138/138)	97.3 to 100.0%
	Pos Control	100.0% (30/30)	27.5	1.30%	100.0% (5/5)	27.5	1.20%	100.0% (5/5)	27	0.80%	100.0% (40/40)	91.2 to 100.0%
	Total Agreement	**100.0% (210/210)**			**100.0% (162/162)**			**100.0% (165/165)**			**100.0% (537/537)**	**98.2 to 100.0%**

[a] Expected Results of HSV-2 Low Positive, HSV-2 Moderate Positive and HSV Negative samples are "Negative" for HSV-1.

		Site – 1			Site – 2			Site – 3				
Target	**Sample**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**Avg. Ct**	**Total %CV**	**% Agreement with Expected Results**	**95% CI**
HSV-2 Result	HSV-1 Low Pos	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Mod Pos	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (30/30) [b]	NA	NA	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Pos	100.0% (30/30)	36.8	3.10%	100.0% (29/29)	37.8	2.30%	100.0% (30/30)	36.6	1.90%	100.0% (89/89)	95.9 to 100.0%
	HSV-2 Mod Pos	100.0% (30/30)	35.2	1.30%	100.0% (30/30)	35.95	1.60%	100.0% (30/30)	34.6	2.30%	100.0% (90/90)	95.9 to 100.0%
	HSV Neg	100.0% (60/60) [b]	NA	NA	100.0% (38/38) [b]	NA	NA	100.0% (40/40) [b]	NA	NA	100.0% (138/138)	95.9 to 100.0%
	Pos Control	100.0% (30/30)	27	1.30%	100.0% (5/5)	27.4	1.50%	100.0% (5/5)	26.8	1.40%	100.0% (40/40)	95.9 to 100.0%
	Total Agreement	**100.0% (210/210)**			**100.0% (162/162)**			**100.0% (165/165)**			**100.0% (537/537)**	**95.9 to 100.0%**

[b] Expected Results of HSV-1 Low Positive, HSV-1 Moderate Positive and HSV Negative samples are "Negative" for HSV-2.

Target	Sample	Site – 1			Site – 2			Site – 3			% Agreement with Expected Results	95% CI
		% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV	% Agreement with Expected Results	Avg. Ct	Total %CV		
IC Result	HSV-1 Low Pos	100.0% (30/30)	30.4	3.80%	100.0% (30/30)	30.3	2.00%	100.0% (30/30)	30.2	1.70%	100.0% (90/90)	95.9 to 100.0%
	HSV-1 Mod Pos	100.0% (30/30)	30.2	2.30%	100.0% (30/30)	30.4	2.80%	100.0% (30/30)	30.1	0.90%	100.0% (90/90)	95.9 to 100.0%
	HSV-2 Low Pos	100.0% (30/30)	29.9	0.50%	100.0% (29/29)	30.4	2.20%	100.0% (30/30)	30.2	0.60%	100.0% (89/89)	95.9 to 100.0%
	HSV-2 Mod Pos	100.0% (30/30)	29.7	0.80%	100.0% (30/30)	30.4	1.20%	100.0% (30/30)	30.1	0.60%	100.0% (90/90)	95.9 to 100.0%
	HSV Neg	100.0% (60/60)	30.2	1.10%	100.0% (40/40)	30.2	1.90%	100.0% (38/38)	30.1	0.90%	100.0% (138/138)	97.3 to 100.0%
	Pos Control	100.0% (30/30)	29.3	1.40%	100.0% (5/5)	30.2	2.10%	100.0% (5/5)	29.4	0.90%	100.0% (40/40)	91.2 to 100.0%
	Total Agreement	**100.0% (210/210)**			**100.0% (164/164)**			**100.0% (163/163)**			**100.0% (537/537)**	**98.2 to 100.0%**

The highest HSV 1&2 ELITe MGB Site-to-Site variability (as measured by %CV based on C_T values) is 2.19%; the highest Lot-to-Lot is 0.23%, and the highest Operator-to-Operator variability is 0.93% for Moderate Positive panel members.

B. Limit of Detection (LoD) / Analytical Sensitivity

The Limit of detection of the HSV 1&2 ELITe MGB Assay was evaluated using commercially available quantitated HSV positive isolates (two HSV-1 and two HSV-2) indicated in Table 13, below. The isolates were diluted in UTM to 100 $TCID_{50}$/mL and then further diluted using 1:3 dilutions in UTM. The LoD results were determined using Probit (Logit) Data Analysis software (Analyse-it for Microsoft Excel v4.80.2, Logistic Function model). LoD was determined as the lowest concentration of the HSV target that can be consistently detected in ≥95% of samples tested under routine laboratory conditions in mucocutaneous swab specimen. The LoD was confirmed by testing twenty (20) additional replicates at the LoD concentration and demonstrating that the virus was detected 95% of the time.

Table 13: List of HSV LoD Isolates & Results

Organism	Isolate/Strain	Cell Line	Qualitative results #detected/Total	Mean C_T ±SD from detected replicates	1×LoD $TCID_{50}$/mL
HSV-1	MacIntyre strain	Vero	20/20	37.91 ± 0.69	59.0 $TCID_{50}$/mL
HSV-1	Isolate #15 (ZeptoMetrix)	Vero	20/20	39.94 ± 0.95	1.5 $TCID_{50}$/mL
HSV-2	MS strain	Vero	20/20	37.90 ± 0.92	5.4 $TCID_{50}$/mL
HSV-2	Isolate #2 (ZeptoMetrix)	Vero	20/20	38.67 ± 1.03	0.3 $TCID_{50}$/mL

The limit of Blank (LoB) was also confirmed to be zero for both the HSV-1 and HSV-2 targets using 60 replicates of HSV negative pooled human cheek matrix.

The elution efficiency of the Copan regular flocked swab in $TCID_{50}$/swab units was determined to be ~100% and directly proportional to the $TCID_{50}$/mL depending only on the volume of the media in the collection device.

C. Assay Cut Off

The assay cut-off analysis was performed on a separate set of 141 clinical samples collected from 3 clinical sites. Each clinical sample was evaluated using HSV 1&2 ELITe MGB Assay in conjunction with the ELITe InGenius instrument and a composite reference method (FDA-cleared real-time PCR assay combined with PCR amplification and bi-directional sequencing). Both targets in clinical samples were detected up to cycle 45. Therefore C_T of 45 was established as a diagnostic assay cut-off for both HSV-1 and HSV-2 targets.

D. Analytical Reactivity (Inclusivity)

The analytical reactivity (Inclusivity) was evaluated by preparing 44 commercially available quantitated HSV-1 or HSV-2 isolates (22 HSV-1 and 22 HSV-2) indicated in Table 14, below. Each isolate was diluted in

UTM to 3×LoD concentration (177 $TCID_{50}$/mL for HSV-1 and 16.2 $TCID_{50}$/mL for HSV-2) and then evaluated using HSV 1&2 ELITe MGB Assay in conjunction with the ELITe InGenius instrument. All of the HSV-1 and HSV-2 tested isolates in the table below were detected by the HSV 1&2 ELITe MGB Assay at concentrations of 16.2 – 354 $TCID_{50}$/mL.

Table 14: Summary of HSV Analytical Reactivity (Inclusivity) Results

#	Isolate	Estimated 1×LoD ($TCID_{50}$/mL)	×LoD Tested	Final Test Conc. ($TCID_{50}$/mL)	Positivity
1	HSV-1 MacIntyre Strain	59	3×	177	3/3
2	HSV-1 Isolate #1	59	3×	177	3/3
3	HSV-1 Isolate #2	59	3×	177	3/3
4	HSV-1 Isolate #3	59	3×	177	3/3
5	HSV-1 Isolate #4	59	3×	177	3/3
6	HSV-1 Isolate #5	59	3×	177	3/3
7	HSV-1 Isolate #6	59	3×	177	0/3
		59	6×	354	3/3
8	HSV-1 Isolate #7	59	3×	177	3/3
9	HSV-1 Isolate #8	59	3×	177	3/3
10	HSV-1 Isolate #9	59	3×	177	3/3
11	HSV-1 Isolate #10	59	3×	177	3/3
12	HSV-1 Isolate #11	59	3×	177	3/3
13	HSV-1 Isolate #12	59	3×	177	3/3
14	HSV-1 Isolate #13	59	3×	177	3/3
15	HSV-1 Isolate #14	59	3×	177	3/3
16	HSV-1 Isolate #15	59	3×	177	3/3
17	HSV-1 Isolate #16	59	3×	177	3/3
18	HSV-1 Isolate #17	59	3×	177	3/3
19	HSV-1 Isolate #18	59	3×	177	3/3
20	HSV-1 Isolate #19	59	3×	177	3/3
21	HSV-1 Isolate #20	59	3×	177	0/3
		59	6×	354	3/3
22	HSV-1 Isolate #21	59	3×	177	3/3
23	HSV-2 MS Strain	5.4	3×	16.2	3/3
24	HSV-2 Isolate #1	5.4	3×	16.2	3/3
25	HSV-2 Isolate #2	5.4	3×	16.2	3/3
26	HSV-2 Isolate #3	5.4	3×	16.2	3/3
27	HSV-2 Isolate #4	5.4	3×	16.2	3/3
28	HSV-2 Isolate #5	5.4	3×	16.2	3/3
29	HSV-2 Isolate #6	5.4	3×	16.2	3/3
30	HSV-2 Isolate #7	5.4	3×	16.2	3/3
31	HSV-2 Isolate #8	5.4	3×	16.2	2/3
		5.4	3×	16.2	3/3
32	HSV-2 Isolate #9	5.4	3×	16.2	3/3
33	HSV-2 Isolate #10	5.4	3×	16.2	3/3
34	HSV-2 Isolate #11	5.4	3×	16.2	2/3
		5.4	6×	32.4	3/3
35	HSV-2 Isolate #12	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3
36	HSV-2 Isolate #13	5.4	3×	16.2	0/3
		5.4	6×	32.4	2/3
		5.4	12×	64.8	2/3
		5.4	24×	129.6	3/3
37	HSV-2 Isolate #14	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3

#	Isolate	Estimated 1×LoD ($TCID_{50}$/mL)	×LoD Tested	Final Test Conc. ($TCID_{50}$/mL)	Positivity
38	HSV-2 Isolate #15	5.4	3×	16.2	0/3
		5.4	6×	32.4	3/3
39	HSV-2 Isolate #16	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3
40	HSV-2 Isolate #17	5.4	3×	16.2	1/3
		5.4	6×	32.4	1/3
		5.4	12x	64.8	3/3
41	HSV-2 Isolate #18	5.4	3×	16.2	3/3
42	HSV-2 Isolate #19	5.4	3×	16.2	2/3
		5.4	6×	32.4	1/3
		5.4	12×	64.8	3/3
43	HSV-2 Isolate #20	5.4	3×	16.2	0/3
		5.4	6×	32.4	1/3
		5.4	12×	64.8	3/3
44	HSV-2 Isolate #21	5.4	3×	16.2	3/3

E. Analytical Specificity (Cross-Reactivity)

Potential cross-reactivity of the HSV 1&2 ELITe MGB Assay was evaluated by testing organisms that are closely related to HSV or cause similar clinical symptoms or may be present in the anogenital and oral cutaneous and mucocutaneous sites tested by this device. 49 potential cross reactants were evaluated. For each organism, the sample to be tested was prepared from quantified stock diluted to the required concentration using Universal Transport Media (UTM). The potential cross reactants tested, the concentrations evaluated, and the results are presented in table 15 below:

Table 15: Cross-Reactivity Test Results:

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
1	*Acinetobacter calcoaceticus*	1×10^6 CFU/mL	0/3	0/3
2	*Acinetobacter lwoffi*	1×10^6 CFU/mL	0/3	0/3
3	Adenovirus type 2	1×10^5 $TCID_{50}$/mL	0/3	0/3
4	*Bacteroides fragilis*	1×10^6 CFU/mL	0/3	0/3
5	*Candida albicans*	1×10^6 CFU/mL	0/3	0/3
6	*Candida glabrata*	1×10^6 CFU/mL	0/3	0/3
7	*Candida guilliermondii*	1×10^6 CFU/mL	0/3	0/3
8	*Candida krusei*	1×10^6 CFU/mL	0/3	0/3
9	*Candida lustaniae*	1×10^6 CFU/mL	0/3	0/3
10	*Candida parapsilosis*	1×10^6 CFU/mL	0/3	0/3
11	*Candida tropicalis*	1×10^6 CFU/mL	0/3	0/3
12	*Chlamydia trachomatis*	1×10^6 CFU/mL	0/3	0/3
13	Cytomegalovirus	1×10^5 $TCID_{50}$/mL	0/3	0/3
14	*Enterobacter cloacae*	1×10^6 CFU/mL	0/3	0/3
15	Enterovirus	1×10^5 $TCID_{50}$/mL	0/3	0/3
16	Epstein-Barr Virus	1×10^5 $TCID_{50}$/mL	0/3	0/3
17	*Escherichia coli*	1×10^6 CFU/mL	0/3	0/3
18	*Fusobacterium nucleatum*	1×10^6 CFU/mL	0/3	0/3
19	*Gardnerella vaginalis*	1×10^6 CFU/mL	0/3	0/3
20	*Haemophilus ducreyi*	1×10^6 CFU/mL	0/3	0/3
21	Human Genomic DNA	500 ng/swab	0/3	0/3
22	Human Herpes Virus 6	1×10^5 $TCID_{50}$/mL	0/3	0/3
23	Human Herpes Virus 7	1×10^5 $TCID_{50}$/mL	0/3	0/3
24	Human papilloma virus 16	1×10^5 $TCID_{50}$/mL	0/3	0/3

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV-1	HSV-2
25	Human papilloma virus 18	1×10^5 $TCID_{50}$/mL	0/3	0/3
26	Herpes Simplex Virus 1 (HSV-1), isolate 20, ZMC	1×10^5 $TCID_{50}$/mL	3/3	0/3
27	Herpes Simplex Virus 2 (HSV-2), isolate 20. ZMC	1×10^5 $TCID_{50}$/mL	0/3	3/3
28	*Klebsiella pneumoniae*	1×10^6 CFU/mL	0/3	0/3
29	*Lactobacillus acidophilus*	1×10^6 CFU/mL	0/3	0/3
30	*Mobiluncus curtsii*	1×10^6 CFU/mL	0/3	0/3
31	*Mobiluncus mulieris*	1×10^6 CFU/mL	0/3	0/3
32	*Moraxella catarrhalis*	1×10^6 CFU/mL	0/3	0/3
33	*Mycoplasma hominis*	1×10^6 CFU/mL	0/3	0/3
34	*Neisseria gonorrhea*	1×10^6 CFU/mL	0/3	0/3
35	*Neisseria meningitides*	1×10^6 CFU/mL	0/3	0/3
36	*Prevotella melaninogenica*	1×10^6 CFU/mL	0/3	0/3
37	Rubella Virus	1×10^5 $TCID_{50}$/mL	0/3	0/3
38	*Staphylococcus aureus (*MSSA)	1×10^6 CFU/mL	0/3	0/3
39	*Staphylococcus epidermidis* (MRSE)	1×10^6 CFU/mL	0/3	0/3
44	*Staphylococcus saprophyticus*	1×10^6 CFU/mL	0/3	0/3
41	*Streptococcus mitis*	1×10^6 CFU/mL	0/3	0/3
42	*Streptococcus mutans*	1×10^6 CFU/mL	0/3	0/3
43	*Streptococcus pneumoniae*	1×10^6 CFU/mL	0/3	0/3
44	*Streptococcus pyogenes*	1×10^6 CFU/mL	0/3	0/3
45	*Streptococcus salivarius*	1×10^6 CFU/mL	0/3	0/3
46	*Toxoplasma gondii*	1×10^6 CFU/mL	0/3	0/3
47	*Trichomonas vaginalis*	1×10^6 CFU/mL	0/3	0/3
48	Varicella-Zoster Virus (VZV)	1×10^5 $TCID_{50}$/mL	0/3	0/3
49	*Chlamydophila pneumoniae*	1×10^6 CFU/mL	0/3	0/3

F. Microbial Interference

The microbial interference was evaluated in the presence of either HSV-1 or HSV-2 spiked at 3×LoD in UTM and the 49 organisms indicated in Table 15, above. Each microorganism was tested either at 1×10^6 CFU/mL or higher for bacterial isolates, or at 1×10^5 $TCID_{50}$/mL or higher for viruses. None of the non-target organisms that are reasonably expected to be found in typical cutaneous and mucocutaneous swab samples interfered with the detection of HSV-1 or HSV-2 species.

G. Competitive Interference of HSV-1 and HSV-2

Competitive interference was studied to evaluate the effects of possible clinically relevant co-infection with both HSV-1 and HSV-2 using HSV 1&2 ELITe MGB Assay.

The study assessed whether a high concentration of one virus in the sample could potentially affect the HSV 1&2 ELITe MGB Assay performance for the other target present at low levels. A low positive sample was contrived at approximately 3×LoD for each target (HSV-1 MacIntyre strain and HSV-2 MS strain), and a baseline Ct was determined for each sample. Each potential concomitant infecting virus was spiked into the low level sample and assayed in triplicate. Competitive interference of HSV-2 was observed at 1×10^5, 1×10^4, and 1×10^3 HSV-2 levels. No competitive interference of HSV-1 was observed at all the levels. The results of the testing are shown in the table below.

Table 16: Competitive Interference of HSV-1 and HSV-2 targets in unequal concentrations

Baseline (Low Level)		Competitive Interferent (High Concentration)		Qualitative Results (#Detected/#Total)	
Strain	Concentration ($TCID_{50}$/mL)	Strain	Concentration ($TCID_{50}$/mL)	HSV-1	HSV-2
HSV-1 MacIntyre	177	HSV-2 MS	1×10^5	0/3	3/3

HSV-1 MacIntyre	177	HSV-2 MS	1×10^4	1/3	3/3
HSV-1 MacIntyre	177	HSV-2 MS	1×10^3	1/3	3/3
HSV-1 MacIntyre	177	HSV-2 MS	1×10^2	3/3	3/3
HSV-1 MacIntyre	177	HSV-2 MS	0	3/3	0/3
HSV-2 MS	16.2	HSV-1 MacIntyre	1×10^5	3/3	3/3
HSV-2 MS	16.2	HSV-1 MacIntyre	1×10^4	3/3	3/3
HSV-2 MS	16.2	HSV-1 MacIntyre	1×10^3	3/3	3/3
HSV-2 MS	16.2	HSV-1 MacIntyre	1×10^2	3/3	3/3
HSV-2 MS	16.2	HSV-1 MacIntyre	0	0/3	3/3

Additionally, in a separate study both strains were tested at similar or equal concentrations of 3×LoD, 1×10^3 and 1×10^5, and no competitive interference was observed.

Table 17: Competitive Interference of HSV-1 and HSV-2 targets in equal concentrations

HSV-1 Concentration		HSV-2 Concentration		Qualitative Results (#Detected/#Total)		Quantitative Results (%CV)	
Strain	Concentration ($TCID_{50}$/mL)	Strain	Concentration ($TCID_{50}$/mL)	HSV-1	HSV-2	HSV-1	HSV-2
HSV-1 MacIntyre	1×10^5	HSV-2 MS	1×10^5	5/5	5/5	3.02 %	1.64 %
HSV-1 MacIntyre	1×10^3	HSV-2 MS	1×10^3	5/5	5/5	1.09 %	2.95 %
HSV-1 MacIntyre	177 (3×LoD)	HSV-2 MS	16.2 (3×LoD)	5/5	5/5	1.74 %	1.88 %

H. Interfering Substances

The performance of the HSV 1&2 ELITe MGB Assay was evaluated with potentially interfering substances that could be encountered in lesion swab specimens obtained from cutaneous and mucocutaneous locations. The substance interference was evaluated in the presence of either HSV-1 or HSV-2 spiked at 3×LoD in UTM for 33 potentially interfering substances at concentrations indicated in Table 18 below. Each panel member was tested in triplicate. No interference with HSV-1 or HSV-2 detection was observed.

Table 18: Interfering Substances Test Panel

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV-1	HSV-2	IC
Whole blood with EDTA	5% v/v	0/3	0/3	3/3
Buffy coat	5% v/v	0/3	0/3	3/3
Acyclovir	2.5 mg/mL	0/3	0/3	3/3
Albumin	5 mg/mL	0/3	0/3	3/3
Casein	7 mg/mL	0/3	0/3	3/3
Female urine	10% v/v	0/3	0/3	3/3
Male urine	10% v/v	0/3	0/3	3/3
K-Y Brand jelly	5% w/v	0/3	0/3	3/3
Douche	10% v/v	0/3	0/3	3/3
Spermicide	5% w/v	0/3	0/3	3/3
Yeast·Gard	1% w/v	0/3	0/3	3/3
Monistat 1	5% w/v	0/3	0/3	3/3
Monistat 3	5% w/v	0/3	0/3	3/3
Vagisil Cream	1% w/v	0/3	0/3	3/3
Tioconazole 1	5% w/v	0/3	0/3	3/3
Rite Aid Feminine Wash, Sensitive Skin	10% v/v	0/3	0/3	3/3
Clotrimazole-7 vaginal cream	1% w/v	0/3	0/3	3/3
Oral Analgesic Gel	5% w/v	0/3	0/3	3/3

Listerine antiseptic mouthwash	10% v/v	0/3	0/3	3/3
Abreva	10% v/v	0/3	0/3	3/3
Carmex lip balm	1% w/v	0/3	0/3	3/3
Releev cold sore treatment	1% v/v	0/3	0/3	3/3
Lip Clear lysine	1% w/v	0/3	0/3	3/3
Toothpaste	5% w/v	0/3	0/3	3/3
Acetaminophen	5 mg/mL	0/3	0/3	3/3
Wal-Finate	5 mg/mL	0/3	0/3	3/3
Cold-Eeze	7% w/v	0/3	0/3	3/3
Non-GMO Corn Starch	1.25 mg/mL	0/3	0/3	3/3
Zinc Oxide Ointment	7% w/v	0/3	0/3	3/3
Cough DM	10mg/mL	0/3	0/3	3/3
Lanacane Max Strength anti-itch cream	7% w/v	0/3	0/3	3/3
Seminal fluid	7% v/v	0/3	0/3	3/3
Foscarnet sodium	5% v/v	0/3	0/3	3/3

I. Carry-Over and Cross-Contamination

The carry-over and cross-contamination was evaluated with the HSV 1&2 ELITe MGB Assay in conjunction with the ELITe InGenius instrument by testing alternating HSV-1 high positive and HSV-1 and HSV-2 negative samples. No evidence of cross-contamination was found during the study.

Table 19: Carry-Over and Cross-Contamination Results

Run description	Positive Samples		Negative Samples	
	# Neg	% Neg.	# Pos.	% Pos.
Run #1, BLANK	0 / 0	NA	0 / 10	0 %
Run #2, Checkerboard	0 / 5	0 %	0 / 6	0 %
Run #3, Checkerboard	0 / 6	0 %	0 / 6	0 %
Run #4, BLANK	0 / 0	NA	0 / 10	0 %
Run #5, Checkerboard	0 / 6	0 %	0 / 6	0 %
Run #6, Checkerboard	0 / 6	0 %	0 / 6	0 %
Run #7, Checkerboard	0 / 6	0 %	0 / 6	0 %
All runs	0 / 29	0 %	0 / 50	0 %

J. Sample Stability

This study assessed both sample stability and sample freeze-thaw stability. The samples for the stability evaluation were prepared by spiking both the HSV-1 and HSV-2 vendor quantitated viral stocks (HSV-1 MacIntyre strain and HSV-2 MS strain) in UTM, M4, M4RT, M5 and M6 media. Each stability sample set consisted of:

- 5 replicates spiked at 3×LoD,
- 5 replicates spiked at 1×10^3 $TCID_{50}$/mL, and
- 5 replicates spiked at 1×10^5 $TCID_{50}$/mL (15 replicates total for each sample set).

The stability of each sample set was assessed and confirmed by incubation at +4°C for 1 week. All HSV-1 and HSV-2 samples were confirmed to be stable in UTM, M4, M4RT, M5 and M6 media for 1 week at +4°C.

The storage conditions were also validated by re-testing previously analyzed clinical samples that were stored in a -80°C freezer (≤ -70°C) for minimum of 4 months. Sample concentrations covered HSV clinical range. Ten HSV-1 or HSV-2 positive samples were tested for each media (except M6 for which only 7 HSV-positive samples were available). Positivity of all samples was confirmed after 4 month storage in a -80°C freezer (≤ -70°C).

5 sets of samples prepared as above in UTM, M4, M4RT, M5 and M6 media were subjected to 3 freeze-thaw cycles. All the samples were tested with the HSV 1&2 ELITe MGB Assay on the ELITe InGenius. The data obtained show that HSV-1 and HSV-2 viruses are stable after 3 freeze-thaw cycles in UTM, M4, M4RT, M5 and M6 media.

Table 20: Summary of stability data

Media\ Conditions	+4°C (1 week)	-70°C (4 months)	3 freeze-thaw cycles
UTM	+	+	+
M4	+	+	+
M4RT	+	+	+
M5	+	+	+
M6	+	+	+

K. Matrix comparison study

Since all analytical studies were conducted in the UTM (Universal Transport Media) and clinical studies were conducted using UTM, M4, M4RT, M5 and M6 media, the Matrix Comparison Study was performed.

The matrix comparison study was conducted using contrived sample panel made by spiking either HSV-1 or HSV-2 quantitated viral strains into each of the recommended media: UTM, M4, M4RT, M5 and M6. Each sample set consisted of 3 replicates spiked at 3×LoD, 3 replicates spiked at 1×10^3 $TCID_{50}$/mL, and 3 replicates spiked at 1×10^5 $TCID_{50}$/mL (9 replicates total for each sample set). Each sample was processed on the InGenius using the HSV 1&2 ELITe MGB Assay. All replicates in all media were detected and showed comparable results. The results of media comparison are shown in Table 21 below:

Table 21. Matrix comparison study results

Target/ Channel	Sample Titer $TCID_{50}$/mL	Sample Matrix					All Media Avg C_T	All Media StDev	All Media %CV
		UTM, Avg C_T	M4, Avg C_T	M4RT, Avg C_T	M5, Avg C_T	M6, Avg C_T			
HSV-2 CH1, FAM	1.00E+05	27.15	26.76	26.42	26.82	27.23	26.88	0.33	1.21%
	1.00E+03	33.86	33.59	33.76	33.51	34.15	33.77	0.25	0.74%
	3×LoD	36.32	35.56	35.96	35.54	36.14	35.91	0.35	0.97%
HSV-1 CH4, AP593	1.00E+05	22.02	21.13	20.82	20.77	20.63	21.08	0.56	2.66%
	1.00E+03	28.01	28.47	27.97	28.58	26.72	27.95	0.74	2.64%
	3×LoD	35.84	36.07	37.02	35.43	34.69	35.81	0.86	2.39%

All tested media showed comparable performance and can be recommended for sample collection and testing with HSV 1&2 ELITe MGB Assay.

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SYMBOLS

REF	Catalog number	IVD	*In vitro* diagnostic medical device
	Upper temperature limit		Contains sufficient material for <n> tests
LOT	Batch code		Consult instructions for use
	Use by		Manufacturer
	For prescription use only		Warning
	Positive Control		Control
	Negative Control		Keep away from sunlight
CONT	Contents		

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