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EMPOWERING IVD

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NOTICE of CHANGE dated 04/05/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HSV1/2 ELITe MGB® Kit»
Ref. RTK403ING

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Change of the Legal Manufacturer.*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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HSV 1&2 ELITe MGB® Kit

reagents for DNA Real-Time PCR Amplification

REF RTK403ING CE IVD -20°C

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INTENDED USE

The **«HSV 1&2 ELITe MGB® Kit»** is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the detection and differentiation of Herpes Simplex Virus 1 and 2 (HSV1 and HSV2) DNA in cutaneous or mucocutaneous lesion swab specimens from patients with signs and symptoms of HSV1 or HSV2 infection.

The product is intended as an aid in the differential diagnosis of HSV1 and HSV2 infections together with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLES

The assay consists of a multiplex real time amplification reaction performed by **ELITe InGenius**, an automated integrated system for extraction, amplification, detection and results interpretation.

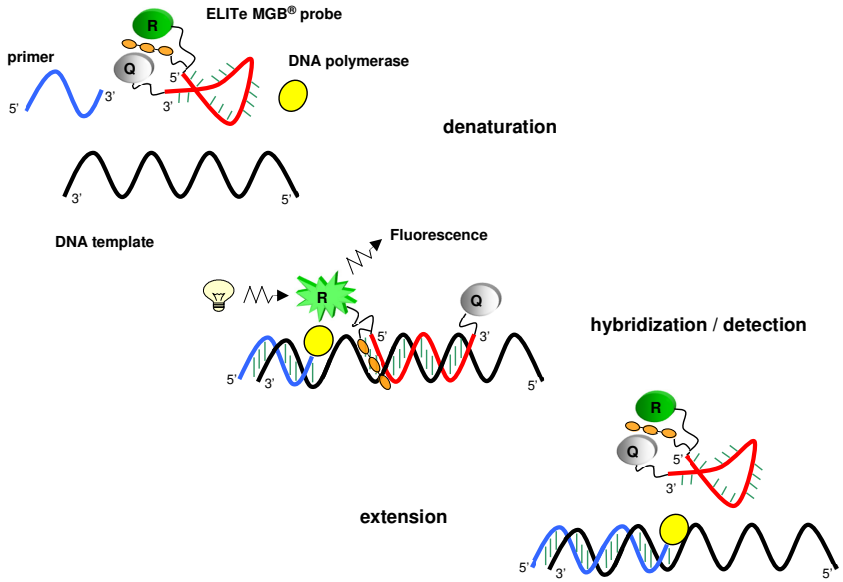
Starting from DNA extracted from each sample under test, different amplification reactions are performed by the **HSV 1&2 PCR mix** in the PCR Cassette in order to amplify the following targets:

- **HSV1** (glycoprotein D gene), detected by the specific probe in the Channel **HSV1** of the ELITe InGenius system (Channel 4). The HSV1 specific probe is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.
- **HSV2** (glycoprotein G gene), detected by the specific probe in the Channel **HSV2** of the ELITe InGenius system (Channel 1). The HSV2 specific probe is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.
- **Internal Control** (artificial sequence IC2), detected by the specific probe in the Channel **IC** of the ELITe InGenius system (Channel 2). The IC specific probe is labelled with AP525 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

The probes with ELITe MGB® technology are activated when they hybridize with the specific product of the amplification reaction. The fluorescence emission is measured and recorded by the instrument. At the end of amplification cycle, the fluorescence plots are analysed to identify the threshold cycles (Ct). The result interpretation allows to detect the presence of the pathogens of interest.

The assay has been validated with **ELITe InGenius®**, automated integrated system for extraction, amplification and detection of nucleic acids.

In the following picture is synthetically showed the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolysed during the amplification cycle so as it can be used for the dissociation curve analysis.



PRODUCT DESCRIPTION

The **«HSV 1&2 ELITe MGB® Assay»** provides the following components:

- **HSV 1&2 PCR Mix**

A mixture of primer oligonucleotides and probes for Real Time amplification, in a stabilizing solution, aliquoted into eight test tubes (YELLOW cap). Each tube contains **280 µL** of solution, sufficient for **12 tests** (processing at least 2 samples per session) in association with ELITe InGenius.

- **HSV 1&2 Negative Control**

DNase and RNase-free water, pre-aliquoted into two test tubes (WHITE cap). Each tube contains **1800 µL** of water, sufficient for **8 sessions** (processing 200 µL in an integrated session) in association with **ELITe InGenius**.

- **HSV 1&2 Positive Control**

A buffered solution containing the plasmid DNA templates for HSV1 and HSV2, pre-aliquoted into two test tubes (RED cap). Each tube contains **1800 µL** of solution, sufficient for **8 sessions** (processing 200 µL in an integrated session) in association with **ELITe InGenius**.

- **HSV 1&2 Internal Control**

A buffered solution containing recombinant M13 phage with the artificial sequence IC2 cloned into phage genome, pre-aliquoted into eight test tubes (NEUTRAL cap). Each tube contains **160 µL** of solution, sufficient for **12 extractions** (processing 10 µL per session) in association with **ELITe InGenius**.

- **Sample Dilution Buffer**

The Sample Dilution Buffer is intended to be used for sample dilution when samples in test are too concentrated, as indicated by the ELITe InGenius software (Error 30103). The Sample Dilution Buffer is a buffered solution, pre-aliquoted into eight test tubes (BROWN cap). Each tube contains **1000 µL** of solution.

The kit is sufficient for **96 tests in association with ELITe InGenius,** including controls.

MATERIALS PROVIDED IN THE PRODUCT

Box	Component	Description	Quantity	Classification of hazards
1	**HSV 1&2 PCR Mix**	reaction mix for HSV1&2 **YELLOW cap**	**8 x 280 µL**	-
	HSV 1&2 Negative Control	DNase and RNase-free water **WHITE cap**	**2 x 1800 µL**	-
2	**HSV 1&2 Internal Control**	recombinant phage solution **NEUTRAL cap**	**8 x 160 µL**	-
3	**HSV 1&2 Positive Control**	plasmid DNA solution **RED cap**	**2 x 1800 µL**	-
	Sample Dilution Buffer	buffer solution **BROWN cap**	**8 x 1000 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood or Biological Safety Cabin.
- 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).

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OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples to be analyzed and the consumables are **not** included in this product.

For automatic DNA extraction, Real Time PCR and result interpretation of samples, the **«ELITe InGenius»** instrument (ELITechGroup S.p.A., ref. INT030-K) and the following specific Assay Protocols (ELITechGroup S.p.A.) are required:

- parameters for the extraction and amplification positive control **«HSV ELITe_PC»,**
- parameters for the extraction and amplification negative control **«HSV ELITe_NC»,**
- parameters for samples to be run and analyzed **«HSV ELITe_MCS_200_50».**

With the **«ELITe InGenius»** instrument the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200),
- consumables for extraction and amplification **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR),
- tips **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in the work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Refer to the current version of IFU available online.
Carefully read all the instructions provided with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

Lab coats, gloves and tools dedicated to work session setup are needed.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases and free from DNA and RNA.

The PCR Cassettes must be handled in such a way to reduce as much as possible amplification product diffusion into the environment in order to avoid sample and reagent contamination.

Warnings and precautions specific for the components

- **HSV1 &2 PCR Mix**

The **HSV 1&2 PCR Mix** must be stored at -20 °C in the dark.
The **HSV 1&2 PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

- **HSV 1&2 Negative Control**

The **HSV1&2 ELITe Negative Control** must be stored at -20 °C.

- **HSV 1&2 Positive Control**

The **HSV 1&2 Positive Control** must be stored at -20 °C.
The **HSV 1&2 Positive Control** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

- **HSV 1&2 Internal Control**

The **HSV 1&2 Internal Control** must be stored at -20 °C.
The **HSV 1&2 Internal Control** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

- **Sample Dilution Buffer**

The **Sample Dilution Buffer** must be stored at -20 °C.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Cutaneous and mucocutaneous lesion swab samples

The cutaneous and mucocutaneous lesion swab samples for DNA extraction must be collected and stored in UTM, M4, M4RT, M5 or M6 viral transport media and identified according to laboratory guidelines. The specimens must be transported and stored in a refrigerator (+2 / +8 °C) for a maximum of 7 days or at -70 °C for a maximum of 3 months.

Split the samples into aliquots before freezing, in order to prevent repeated cycles of freezing and thawing.

Store purified nucleic acids at +2 / +8 °C if they will be used on the same day they were extracted or at -20 °C for long term storage.

Note: to carry out the DNA extraction from mucocutaneous lesion swab samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol: **HSV ELITe_MCS_200_50**. This protocol processes 200 µL of sample, adds 10 µL of **HSV 1&2 Internal Control** per extraction and elutes the nucleic acids in 50 µL.

Samples provided in an ELITe InGenius compatible primary tube (12×80 mm or 13x100 screw cap tube with internal conical shape, Copan Italia S.p.A., or similar) with a sample volume of at least 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 lower than 2.2 mL require a 200 µL aliquot to be transferred into a Sonication tube placed in the ELITe InGenius Sonication tube rack. Refer to the ELITe InGenius Operator's Manual (SCH mINT030_en) for more information.

Extraction and Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the extraction and amplification controls for the amplification reagent lot that will be used in testing:

as a Positive Control, use the **HSV 1&2 Positive Control** product provided with this kit, in association with protocol **HSV ELITe_PC**,

as a Negative Control, use the **HSV 1&2 Negative Control** product provided with this kit, in association with protocol **HSV ELITe_NC**.

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Note: The **ELITe InGenius** system requires approved and valid results of extraction and amplification controls for each amplification reagent lot stored in its database.
The control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot in use.
Furthermore, the extraction and amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality controls (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used.

PROCEDURE

The procedure to use the **HSV 1&2 ELITe MGB® Kit** with the system **ELITe InGenius** consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and approval of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe InGenius** instrument and select the login mode "**CLOSED**",
- verify that the extraction and amplification controls (Controls, HSV 1&2 Positive Control, HSV 1&2 Negative Control) were run in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are no extraction and amplification control results approved or valid, generate them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, the **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **HSV 1&2 ELITe MGB® Kit** is described in the table below:

Assay Protocol for HSV 1&2 ELITe MGB® Kit			
Name	**Matrix**	**Report**	**Characteristics**
HSV ELITe_MCS_200_50	cutaneous and mucocutaneous lesion swab samples	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The product **HSV 1&2 ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run (PCR only),
C. Positive Control and Negative Control integrated run (Extract + PCR).

All the parameters needed for the session are included in the Assay Protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: The ELITe InGenius system can be linked to the "Laboratory Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the three types of run are described here below.

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw HSV 1&2 PCR Mix tube for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 samples per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw HSV 1&2 PCR Mix in the dark because the reagent is sensitive to the light.

2. Thaw the HSV 1&2 Internal Control tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL.
5. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
6. Select the Assay protocol to be used in the “Assay” column (i.e. HSV ELITe_MCS_200_50).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position in the “Sample Position” column:
   - if a primary tube is used, select “Primary Tube”,
   - if a secondary tube is used, select “Sonication Tube”.

   Click “Next” to continue the setup.
9. Load HSV 1&2 PCR Mix and HSV 1&2 Internal Control on the “Inventory Block” selected by following the GUI instruction.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” button to continue the setup.
11. Load the “PCR Cassettes”, the “ELITe InGenius SP 200” extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve and store the results and to print and save the report.

Note: At the end of the run, the remaining unextracted sample in the “Primary tube” must be removed from the instrument, capped and stored. Avoid spilling the unextracted sample.

Note: At the end of the run, the remaining extracted sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C. Avoid spilling the extracted sample.

Note: At the end of the run, the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR Mix can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time required to setup a third work session.

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B. Amplification run

To set up an amplification run starting from extracted Nucleic Acids, carry out the following steps as per GUI:

1. Thaw HSV 1&2 PCR Mix tube for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw HSV 1&2 PCR Mix in the dark because these reagents are sensitive to the light.

2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay protocol to be used in the “Assay” column (i.e. HSV ELITe_MCS_200_50).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load HSV 1&2 PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassettes” and the extracted Nucleic Acids samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve and store the results and to print and save the report.

Note: At the end of the run the remaining extracted sample in the “Elution tube” must be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the extracted sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR mix can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time required to setup a third work session.

C. Positive Control and Negative Control integrated run

To setup the integrated run with Positive Control and Negative Control extraction and amplification, carry out the following steps as per GUI:

1. Thaw HSV 1&2 PCR Mix tube for the session. Each tube is sufficient for preparing 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw HSV 1&2 PCR Mix in the dark because these reagents are sensitive to the light.

2. Thaw the HSV 1&2 Internal Control tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Thaw the HSV 1&2 Positive Control tube for the session. Each tube is sufficient for 8 sessions. Mix gently, spin down the content for 5 seconds and transfer 200 µL of HSV 1&2 Positive Control in a "Sonication tube".
4. Thaw the HSV 1&2 Negative Control tube for the session. Each tube is sufficient for 8 sessions. Mix gently, spin down the content for 5 seconds and transfer 200 µL of HSV 1&2 Positive Control in a "Sonication tube".
5. Select "Perform Run" from the "Home" screen.
6. Ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.
7. In the Tracks of interest, select the Assay protocol to be used in the "Assay" column.
8. For the Positive Control, select HSV ELITe_PC, in the "Assay" column and fill in the lot number and expiry date of HSV1&2 Positive Control.
9. For the Negative Control, select HSV ELITe_NC, in the "Assay" column and fill in the lot number and expiry date of HSV1&2 Negative Control.
10. Ensure that the "Protocol" displayed is: "Extract + PCR".
11. Ensure that the "Sample Position" displayed is: "Sonication Tube". Click "Next" to continue the setup.
12. Load HSV 1&2 PCR Mix and HSV 1&2 Internal Control on the "Inventory Block" selected by following the GUI instruction.
13. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
14. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the HSV 1&2 Positive Control and 200 µL of HSV 1&2 Negative Control to be extracted in the Tracks specified in step 7, following the GUI instruction. Click "Next" to continue the setup.
15. Close the instrument door.
16. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve and store the results and to print and save the report.

Note: At the end of the run the remaining extracted Positive Control and Negative Control in the "Elution tube" must be disposed. Avoid spilling the extracted sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR mix can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time required to setup a third work session.

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Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report"). Refer to the instrument user's manual for more details.

Note: the **ELITe InGenius** system can be linked to the "Laboratory Information System" (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user's manual for more details.

The **ELITe InGenius** system generates the results with the product **HSV 1&2 ELITe MGB® Kit** through the following procedure:

A. Validation of Positive Control and Negative Control results,
B. Validation of sample results,
C. Sample result reporting.

A. Validation of Positive Control and Negative Control results

The fluorescence signals emitted by the target probes (**HSV1** and **HSV2** Channels) in the Positive Control and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols "HSV ELITe_PC" and "HSV ELITe_NC".

The Positive Control and Negative Control run results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The Positive Control and Negative Control run results, specific for the amplification reagent lot, will expire **after 15 days**.

The results of Positive Control and Negative Control runs are used by the instrument software to setup the "Control Charts" for monitoring the amplification step performances. Refer to the instrument user's manual for more details.

Note: If the Positive Control or Negative Control run result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case the Positive Control or Negative Control run have to be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the run of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the specific HSV1 and HSV2 probes (**HSV1** and **HSV2** Channels) and by the specific Internal Control probe (**IC** Channel) in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol HSV 1&2 ELITe_MCS_200_50.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are met.

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

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe® InGenius software** algorithm and the Assay Protocol parameters.

The possible result messages are listed in the table below. For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Result of sample run		Interpretation
HSV1 result	**HSV2 result**	
HSV1: DNA Not Detected or below the LoD	HSV2: DNA Not Detected or below the LoD	The **DNA of HSV1 and HSV2 was not detected** in the sample. The sample is negative valid for these pathogens or their concentrations are below the Limits of Detection of the assay.
HSV1: DNA Not Detected or below the LoD	HSV2: DNA Detected	The **DNA of HSV1 was not detected** in the sample. The **DNA of HSV2 was detected** in the sample.
HSV1: DNA Detected	HSV2: DNA Not Detected or below the LoD	The **DNA of HSV1 was detected** in the sample. The **DNA of HSV2 was not detected** in the sample.
HSV1: DNA Detected	HSV2: DNA Detected	The **DNA of HSV1 and HSV2 was detected** in the sample.
Invalid - Retest Sample.		**Invalid assay result** caused by Internal Control failure due to incorrect extraction, inhibitors carry-over. The test should be repeated.

Samples reported as “Invalid - Retest Sample” by the **ELITe InGenius software** are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitors carry-over in the eluate), which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

Samples reported as "HSV1 DNA Not Detected or below the LoD" and "HSV2 DNA Not Detected or below the LoD" are suitable for analysis but it was not possible to detect targets DNA. In this case it cannot be excluded that targets DNA were present at a concentration below the limits of detection of the assay (see “Performance characteristics”).

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

The sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as “Administrator” or “Analyst”, following the GUI instruction. From the “Result Display” window it is possible to print and save the sample run results as “Sample Report” and “Track Report”.

C. Sample result reporting

The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a work session sorted by selected sample (SID).

The “Track Report” shows the details of a work session sorted by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

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PERFORMANCE CHARACTERISTICS

Reproducibility

The reproducibility of the HSV 1&2 ELITe MGB® Kit was evaluated in a multi-site investigation using contrived clinical samples. HSV test panels were prepared by spiking HSV1 (MacIntyre strain) or HSV2 (MS strain) virus into UTM (Universal Transport Media) at the concentrations of <1× LoD, 1 × LoD and 3 × LoD. HSV1 and HSV2 negative panel members were included as panel member controls. The reproducibility panel composition is shown in the table below:

Reproducibility Panel

Name	Description of Contents	Viral Load	Expected Positivity Rate
M1	HSV1 C_{50} (High Negative) in UTM	<1× LOD	20-80% positive
M2	HSV1 C_{95} (Low Positive) in UTM	1× LOD	≥95% positive
M3	HSV1 C_{100} (Moderate Positive) in UTM	2-3 × LOD	100% positive
M4	HSV2 C_{50} (High Negative) in UTM	<1× LOD	20-80% positive
M5	HSV2 C_{95} (Low Positive) in UTM	1× LOD	≥95% positive
M6	HSV2 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® Kit. Testing was performed on a minimum of 90 (30 per site) replicates per panel member. Lot-to-Lot variability was assessed only at ELITechGroup MDx LLC (internal site) using 3 lots of HSV 1&2 ELITe MGB® Kit. 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.

HSV 1&2 ELITe MGB® Kit Reproducibility Results

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		
HSV1 Result	HSV1 Low Pos	100.0% (30/30)	38.9	1.70%	100.0% (30/30)	38.3	2.10%	100.0% (30/30)	38.0	2.00%	100.0% (90/90)	95.9 to 100.0%
	HSV1 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%
	HSV2 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%
	HSV2 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	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 HSV2 Low Positive, HSV2 Moderate Positive and HSV Negative samples are “Negative” for HSV1.

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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		
HSV2 Result	HSV1 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%
	HSV1 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%
	HSV2 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%
	HSV2 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)	27.0	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 HSV1 Low Positive, HSV1 Moderate Positive and HSV Negative samples are "Negative" for HSV2.

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	HSV1 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%
	HSV1 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%
	HSV2 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%
	HSV2 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® Kit 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.

Limit of Detection (LoD) / Analytical Sensitivity

The Limit of detection of the HSV 1&2 ELITe MGB® Kit was evaluated using commercially available quantitated HSV positive isolates (two HSV1 and two HSV2) 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 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.

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
HSV1	MacIntyre strain	Vero	20/20	37.91 ± 0.69	59.0 $TCID_{50}$/mL
HSV1	Isolate #15 (ZeptoMetrix)	Vero	20/20	39.94 ± 0.95	1.5 $TCID_{50}$/mL
HSV2	MS strain	Vero	20/20	37.90 ± 0.92	5.4 $TCID_{50}$/mL
HSV2	Isolate #2 (ZeptoMetrix)	Vero	20/20	38.67 ± 1.03	0.3 $TCID_{50}$/mL

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The limit of Blank (LoB) was also confirmed to be zero for both the HSV1 and HSV2 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.

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® Kit 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 HSV1 and HSV2 targets.

Analytical Reactivity (Inclusivity)

The analytical reactivity (Inclusivity) was evaluated by preparing 44 commercially available quantitated HSV1 or HSV2 isolates (22 HSV1 and 22 HSV2) indicated in Table 14, below. Each isolate was diluted in UTM to 3×LoD concentration (177 $TCID_{50}$/mL for HSV1 and 16.2 $TCID_{50}$/mL for HSV2) and then evaluated using HSV 1&2 ELITe MGB® Kit in conjunction with the ELITe InGenius instrument. All of the HSV1 and HSV2 tested isolates in the table below were detected by the HSV 1&2 ELITe MGB® Kit at concentrations of 16.2 – 354 $TCID_{50}$/mL.

Summary of HSV Analytical Reactivity (Inclusivity) Results

#	Isolate	Estimated 1×LoD ($TCID_{50}$/mL)	×LoD Tested	Final Test Conc. ($TCID_{50}$/mL)	Positivity
1	HSV1 MacIntyre Strain	59	3×	177	3/3
2	HSV1 Isolate #1	59	3×	177	3/3
3	HSV1 Isolate #2	59	3×	177	3/3
4	HSV1 Isolate #3	59	3×	177	3/3
5	HSV1 Isolate #4	59	3×	177	3/3
6	HSV1 Isolate #5	59	3×	177	3/3
7	HSV1 Isolate #6	59	3×	177	0/3
		59	6×	354	3/3
8	HSV1 Isolate #7	59	3×	177	3/3
9	HSV1 Isolate #8	59	3×	177	3/3
10	HSV1 Isolate #9	59	3×	177	3/3
11	HSV1 Isolate #10	59	3×	177	3/3
12	HSV1 Isolate #11	59	3×	177	3/3
13	HSV1 Isolate #12	59	3×	177	3/3
14	HSV1 Isolate #13	59	3×	177	3/3
15	HSV1 Isolate #14	59	3×	177	3/3
16	HSV1 Isolate #15	59	3×	177	3/3
17	HSV1 Isolate #16	59	3×	177	3/3
18	HSV1 Isolate #17	59	3×	177	3/3
19	HSV1 Isolate #18	59	3×	177	3/3
20	HSV1 Isolate #19	59	3×	177	3/3
21	HSV1 Isolate #20	59	3×	177	0/3
		59	6×	354	3/3
22	HSV1 Isolate #21	59	3×	177	3/3
23	HSV2 MS Strain	5.4	3×	16.2	3/3
24	HSV2 Isolate #1	5.4	3×	16.2	3/3
25	HSV2 Isolate #2	5.4	3×	16.2	3/3
26	HSV2 Isolate #3	5.4	3×	16.2	3/3
27	HSV2 Isolate #4	5.4	3×	16.2	3/3
28	HSV2 Isolate #5	5.4	3×	16.2	3/3
29	HSV2 Isolate #6	5.4	3×	16.2	3/3
30	HSV2 Isolate #7	5.4	3×	16.2	3/3
31	HSV2 Isolate #8	5.4	3×	16.2	2/3
		5.4	3×	16.2	3/3
32	HSV2 Isolate #9	5.4	3×	16.2	3/3

#	Isolate	Estimated 1×LoD ($TCID_{50}$/mL)	×LoD Tested	Final Test Conc. ($TCID_{50}$/mL)	Positivity
33	HSV2 Isolate #10	5.4	3×	16.2	3/3
34	HSV2 Isolate #11	5.4	3×	16.2	2/3
		5.4	6×	32.4	3/3
35	HSV2 Isolate #12	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3
36	HSV2 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	HSV2 Isolate #14	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3
38	HSV2 Isolate #15	5.4	3×	16.2	0/3
		5.4	6×	32.4	3/3
39	HSV2 Isolate #16	5.4	3×	16.2	1/3
		5.4	6×	32.4	3/3
40	HSV2 Isolate #17	5.4	3×	16.2	1/3
		5.4	6×	32.4	1/3
		5.4	12x	64.8	3/3
41	HSV2 Isolate #18	5.4	3×	16.2	3/3
42	HSV2 Isolate #19	5.4	3×	16.2	2/3
		5.4	6×	32.4	1/3
		5.4	12×	64.8	3/3
43	HSV2 Isolate #20	5.4	3×	16.2	0/3
		5.4	6×	32.4	1/3
		5.4	12×	64.8	3/3
44	HSV2 Isolate #21	5.4	3×	16.2	3/3

Analytical Specificity (Cross-Reactivity)

Potential cross-reactivity of the HSV 1&2 ELITe MGB® Kit 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:

Cross-Reactivity Test Results

No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV1	HSV2
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

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No.	Potential Cross-Reactants	Tested Concentration	Qualitative Result (#Detected/#Total)	
			HSV1	HSV2
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
25	Human papilloma virus 18	1×10^5 $TCID_{50}$/mL	0/3	0/3
26	Herpes Simplex Virus 1 (HSV1), isolate 20, ZMC	1×10^5 $TCID_{50}$/mL	3/3	0/3
27	Herpes Simplex Virus 2 (HSV2), 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

Microbial Interference

The microbial interference was evaluated in the presence of either HSV1 or HSV2 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 HSV1 or HSV2 species.

Competitive Interference of HSV1 and HSV2

Competitive interference was studied to evaluate the effects of possible clinically relevant co-infection with both HSV1 and HSV2 using HSV 1&2 ELITe MGB® Kit.

The study assessed whether a high concentration of one virus in the sample could potentially affect the HSV 1&2 ELITe MGB® Kit performance for the other target present at low levels. A low positive sample was contrived at approximately 3×LoD for each target (HSV1 MacIntyre strain and HSV2 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 HSV2 was observed at 1×10^5, 1×10^4, and 1×10^3 HSV2 levels. No competitive interference of HSV1 was observed at all the levels. The results of the testing are shown in the table below.

Competitive Interference of HSV1 and HSV2 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)	HSV1	HSV2
HSV1 MacIntyre	177	HSV2 MS	1×10^5	0/3	3/3
HSV1 MacIntyre	177	HSV2 MS	1×10^4	1/3	3/3
HSV1 MacIntyre	177	HSV2 MS	1×10^3	1/3	3/3
HSV1 MacIntyre	177	HSV2 MS	1×10^2	3/3	3/3
HSV1 MacIntyre	177	HSV2 MS	0	3/3	0/3
HSV2 MS	16.2	HSV1 MacIntyre	1×10^5	3/3	3/3
HSV2 MS	16.2	HSV1 MacIntyre	1×10^4	3/3	3/3
HSV2 MS	16.2	HSV1 MacIntyre	1×10^3	3/3	3/3
HSV2 MS	16.2	HSV1 MacIntyre	1×10^2	3/3	3/3
HSV2 MS	16.2	HSV1 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.

Competitive Interference of HSV1 and HSV2 targets in equal concentrations

HSV1 Concentration		HSV2 Concentration		Qualitative Results (#Detected/#Total)		Quantitative Results (%CV)	
Strain	Concentration ($TCID_{50}$/mL)	Strain	Concentration ($TCID_{50}$/mL)	HSV1	HSV2	HSV1	HSV2
HSV1 MacIntyre	1×10^5	HSV2 MS	1×10^5	5/5	5/5	3.02 %	1.64 %
HSV1 MacIntyre	1×10^3	HSV2 MS	1×10^3	5/5	5/5	1.09 %	2.95 %
HSV1 MacIntyre	177 (3×LoD)	HSV2 MS	16.2 (3×LoD)	5/5	5/5	1.74 %	1.88 %

Interfering Substances

The performance of the HSV 1&2 ELITe MGB® Kit 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 HSV1 or HSV2 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 HSV1 or HSV2 detection was observed.

Interfering Substances Test Panel

Potential Interferent	Interferent Concentration	#Detected/#Total		
		HSV1	HSV2	IC
Whole blood with EDTA	5% v/v	3/3	3/3	3/3
Buffy coat	5% v/v	3/3	3/3	3/3
Acyclovir	2.5 mg/mL	3/3	3/3	3/3
Albumin	5 mg/mL	3/3	3/3	3/3
Casein	7 mg/mL	3/3	3/3	3/3
Female urine	10% v/v	3/3	3/3	3/3
Male urine	10% v/v	3/3	3/3	3/3
K-Y Brand jelly	5% w/v	3/3	3/3	3/3
Douche	10% v/v	3/3	3/3	3/3
Spermicide	5% w/v	3/3	3/3	3/3
Yeast·Gard	1% w/v	3/3	3/3	3/3
Monistat 1	5% w/v	3/3	3/3	3/3
Monistat 3	5% w/v	3/3	3/3	3/3
Vagisil Cream	1% w/v	3/3	3/3	3/3
Tioconazole 1	5% w/v	3/3	3/3	3/3
Rite Aid Feminine Wash, Sensitive Skin	10% v/v	3/3	3/3	3/3
Clotrimazole-7 vaginal cream	1% w/v	3/3	3/3	3/3
Oral Analgesic Gel	5% w/v	3/3	3/3	3/3

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

Carry-Over and Cross-Contamination

The carry-over and cross-contamination were evaluated with the HSV 1&2 ELITe MGB® Kit in conjunction with the ELITe InGenius instrument by testing alternating HSV1 high positive and HSV1 and HSV2 negative samples. No evidence of cross-contamination was found during the study.

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 %

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 HSV1 and HSV2 vendor quantitated viral stocks (HSV1 MacIntyre strain and HSV2 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 HSV1 and HSV2 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 HSV1 or HSV2 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 months 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® Kit on the ELITe InGenius. The data obtained show that HSV1 and HSV2 viruses are stable after 3 freeze-thaw cycles in UTM, M4, M4RT, M5 and M6 media.

Summary of stability data

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

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 HSV1 or HSV2 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® Kit. All replicates in all media were detected and showed comparable results. The results of media comparison are shown in Table 21 below:

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			
HSV2 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%
HSV1 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® Kit.

Clinical Evaluation

Study Description

To evaluate the clinical performance of the HSV 1&2 ELITe MGB® Kit, 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® Kit. 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® Kit and the Composite Reference Method. Out of the 1,174 tested samples 2 samples were found invalid by the ELITe MGB® Kit and were excluded from the performance analysis tables.

Out of the 1172 remaining samples 1 additional invalid sample result for HSV1 and 2 additional invalid sample results for HSV2 by the composite reference method were removed from the performance analysis tables.

Therefore, for HSV1, 1171 samples analyzed and for HSV2 1170 samples were analyzed.

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Results: Expected Values/Reference Range

HSV 1&2 Prevalence

The observed expected values for HSV1 and HSV2 in the study population using the HSV 1&2 ELITe MGB Kit 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 HSV1 and HSV2 detected by the ELITe MGB® Kit and one of the samples was confirmed positive by the composite reference method.

Cutaneous and Mucocutaneous HSV 1&2 Prevalence by Age and Gender

Gender	Age Group	Total	HSV 1&2 ELITe MGB® Kit HSV1 results		HSV 1&2 ELITe MGB® Kit HSV2 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%**

Cutaneous HSV 1&2 Prevalence by Lesion Source

Lesion Source	Total	HSV 1&2 ELITe MGB® Kit HSV1 results		HSV 1&2 ELITe MGB® Kit HSV2 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%**

Mucocutaneous HSV 1&2 Prevalence by Lesion Source

Lesion Source	Total	HSV 1&2 ELITe MGB® Kit HSV1 results		HSV 1&2 ELITe MGB® Kit HSV2 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%**

Results: Clinical Performance

HSV1 Positive/Negative Percent Agreements (PPA/NPA) - Summary of the Results:

The PPA/NPA performance of HSV 1&2 ELITe MGB® Kit when compared to the Composite Reference Method in detection of HSV1 DNA in cutaneous and mucocutaneous lesions is summarized in the table below:

Summary of HSV1 Results for Valid Cutaneous Lesion Samples (N=545)

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

Summary of HSV1 Results for Valid Mucocutaneous Lesion Samples (N=626)

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

HSV2 PPA/NPA - Summary of the Results:

The Positive Percent Agreement and Negative Percent Agreement (PPA/NPA) performances of HSV 1&2 ELITe MGB® Kit when compared to the Composite Reference Method in detection of HSV2 DNA in cutaneous and mucocutaneous lesions is summarized in the table below:

Summary of HSV2 Results Valid Cutaneous Lesion Samples (N=545)

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

Summary of HSV2 Results for Valid Mucocutaneous Lesion Samples (N=625)

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

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HSV2 Contrived Oral Panel Study

Due to the difficulty in obtaining sufficient HSV2 positive oral samples, testing for HSV2 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 HSV1 and HSV2 at various concentrations (as shown in table below).

HSV Oral Contrived Sample Panel

Level	Sample #	Contrived sample titer	× LoD
Level 1	10	HSV2 Positive @ 5,400 $TCID_{50}$/mL	1,000×LoD
Level 2	10	HSV2 Positive @ 1,080 $TCID_{50}$/mL	200×LoD
Level 3	10	HSV2 Positive @ 216 $TCID_{50}$/mL	40×LoD
Level 4	10	HSV2 Positive @ 43.2 $TCID_{50}$/mL	8×LoD
Level 5	10	HSV2 Positive @ 16.2 $TCID_{50}$/mL	3×LoD
Level 6	10	HSV1 Positive @ 590 $TCID_{50}$/mL	10×LoD
Level 7	15	HSV1/HSV2 Negative Oral Samples	
Total	75		

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

The HSV2 Oral Contrived Panel Study revealed that 49 out of 50 oral HSV2 contrived samples were positive using HSV 1&2 ELITe MGB® Kit (98% detection).

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument will be recorded in the Product Technical File "HSV1&2 ELITe MGB® Kit", FTP RTK403ING.

REFERENCES

J Schiffer et al. (2015) *Principles and Practice of Infectious Diseases* 2: 1713-30
S. Vanjeri et al. (2012) *J Glob Infect Dis*. 4(3): 139-40
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res*. 35: e30

PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: patients' lesion swab samples stored in UTM and M4, M4RT, M5 or M6 medium.

The device performances were not evaluated in cerebrospinal fluid (CSF), urethral, ocular and nasal mucocutaneous lesions samples.

The results obtained with this product depend on an adequate identification, collection, transport storage and processing of the samples. The results obtained with this product depend also on the use of the adequate related products. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the Real Time amplification method used in this product is sensitive to cross-contaminations from the positive samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, cross-contaminations can be avoided only by good laboratory practices and following these instructions for use.

This product must be handled by qualified personnel trained 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.

This product requires the use of work clothes and areas that are suitable for 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.

This product requires the use of special clothing and instruments dedicated to work session setup to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

HSV1 detection was found to be inhibited in the presence of HSV2 DNA titers of 1×10^3 $TCID_{50}$/mL or higher in the analytical interference study.

A negative result obtained with this product means that the target DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the target DNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

Potential interferences caused by particular patient conditions, could cause incorrect results.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the viral genome covered by the product primers and probes may impair detection of HSV1 and/or HSV2 virus DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

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TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and positive control. Check the volumes of PCR Mix and positive control.
Positive control degradation.	Use a new aliquot of positive control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and negative control. Check the volumes of PCR Mix and negative control.
Contamination of the negative control	Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Sample reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
Internal Control degradation.	Use new aliquots of Internal Control.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a "PCR only" session. Repeat the extraction and amplification with a 1:2 dilution in molecular biology grade water of sample in a "Extract + PCR" session.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. If a Ct value is required: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a "PCR only" session or - repeat the extraction with a 1:10 dilution in Sample Dilution buffer of sample in an "Extract + PCR" session.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	In vitro medical dignostic device.
UDI	Unique Device Identification.
CE	Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
CONTROL	Control
CONTROL +	Positive Control
CONTROL -	Negative Control
	Keep away from sunlight.
	Manufacturer.
	Warning
	Country of Manufacture

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NOTICE TO PURCHASER: LIMITED LICENSE

ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 6972339, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529 and EP patent numbers 1430147, 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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