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MOLECULAR DIAGNOSTICS

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NOTICE of CHANGE dated 14/03/19

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«Meningitis Viral ELITe MGB® Panel»
Ref. RTS507ING

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

- *Modification of the indication of the number of reactions to be prepared in excess during preparation of the complete reaction mixture MV PCR Mix.*

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
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

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Meningitis Viral ELITe MGB® Panel

reagent for DNA Real Time amplification

REF RTS507ING CE -20°C

TABLE OF CONTENTS

INTENDED USE

The **«Meningitis Viral ELITe MGB® Panel»** product is part of a qualitative nucleic acids amplification multiplex assay for the detection of the DNA of herpes simplex virus 1 and 2 (HSV1 and HSV2) and varicella zoster virus (VZV) specific gene in cerebrospinal fluid (CSF) samples.

The product is intended for use in the diagnosis of infections with herpes simplex virus 1 & 2 and varicella zoster virus together with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLES

The assay consists of a multiplex Real Time amplification reaction with an automated integrated system for extraction, Real Time amplification and results interpretation.

Starting from DNA extracted from the samples being tested, three amplification reactions specific for the following viruses are performed in the cartridge:

- herpes simplex virus 1 detected by a specific probe revealed in the ELITe InGenius channel "HSV1"
- herpes simplex virus 2 detected by a specific probe revealed in the ELITe InGenius channel "HSV2"
- varicella zoster virus detected by a specific probe revealed in the ELITe InGenius channel "VZV"

Furthermore, the extraction and inhibition Internal Control (IC) is also amplified in the cartridge. The Internal Control is based on an exogenous target (sequences of the murine cytomegalovirus mCMV) and detected by a specific probe revealed in the ELITe InGenius channel "IC".

Probes with the MGB® TaqMan™ technology are activated when they hybridize with the specific product of the amplification reaction and they are hydrolysed by the DNA polymerase thermostable enzyme. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. Data processing allows detection of viral DNA listed above in the starting sample.

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

PRODUCT DESCRIPTION

The **« Meningitis Viral ELITe MGB® Panel»** product provides the following components:

- **MV primer and probe mix**

A mixture of primer oligonucleotides for Real Time amplification, in a stabilizing solution, aliquoted into two test tubes (VIOLET cap). Each tube contains **90 µL** of solution, sufficient for **48 tests** in association with **ELITe InGenius** when performing a number of reactions corresponding to multiples of 4 and with a maximum of 8 sessions.

- **MV buffer mix**

An optimized and stabilized mixture of reagents for Real Time amplification aliquoted into two test tubes (ORANGE cap). Each tube contains **750 µL** of solution, sufficient for **48 tests** in association with **ELITe InGenius** when performing a number of reactions corresponding to multiples of 4 and with a maximum of 8 sessions.

- **MV enzyme**

An optimized and stabilized mixture of enzymes for Real Time amplification**,** pre-aliquoted into two test tubes (YELLOW cap). Each tube contains **60 µL** of solution, sufficient for **48 tests** in association with **ELITe InGenius** when performing a number of reactions corresponding to multiples of 4 and with a maximum of 8 sessions.

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

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
MV primer and probe mix	primer/probe mix for per HSV1, HSV2, VZV and IC (mCMV) (VIOLET cap)	**2 x 90 µL**	-
MV buffer mix	RT-PCR buffer mix (ORANGE cap)	**2 x 750 µL**	-
MV enzyme	RT-PCR enzyme (YELLOW cap)	**2 x 60 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Sarstedt 2.0 mL tube skirted screw-cap (Sarstedt Ref. 72.694.005).

OTHER PRODUCTS REQUIRED

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

For automatic DNA extraction, Real Time amplification and result interpretation of samples to be analyzed, the **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) instrument and the following specific Assay protocols are required:

- parameters for the amplification positive control **«MV ELITe_PC»** (ELITechGroup S.p.A.),
- parameters for the amplification negative control **«MV ELITe_NC»** (ELITechGroup S.p.A.),
- parameters for samples to be analyzed **«MV ELITe_CSF_200_100»** (ELITechGroup S.p.A.).

For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) 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 Universal Fit Filter Tips»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

As template of extraction and inhibition internal control, the generic product **«500-Internal Control»** (ELITechGroup S.p.A., ref. IC500), is required. This is based on murine cytomegalovirus (mCMV).

As template of amplification positive control, the specifc product **«Meningitis Viral-ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR507ING), is required. This is a stabilised solution of plasmid DNAs.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

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 of 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.
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

- **MV primer and probe mix**

The MV primer and probe must be stored at -20 °C in the dark.

The MV primer and probe can be frozen and thawed for no more than **eight times**: further freezing / thawing cycles may cause a loss of product performances.

- **MV enzyme**

The MV enzyme must be stored at -20 °C.

The MV enzyme must not be exposed to temperatures higher than -20 °C for more than 10 minutes. It is recommended to keep it on ice or in the cooling block. It can be used for no more than **eight sessions**.

- **MV buffer mix**

The MV buffer must be stored at -20 °C.

The MV buffer can be frozen and thawed for no more than **eight times**: further freezing / thawing cycles may cause a loss of product performances.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Cerebrospinal fluid (CSF)

The cerebrospinal fluid samples for nucleic acids extraction must be collected according to laboratory guidelines, avoiding contamination by patient blood, transported at +2°/+8°C for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples into aliquots before freezing, in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from CSF is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.1 (or later equivalent versions), use the Assay protocol **MV ELITe_CSF _200_100**. This protocol processes 200 µL of sample, adds the **500 Internal Control** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Amplification controls

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

as a Positive amplification Control, use the **Meningitis Viral-ELITe Positive Control** product (not provided with this kit) in association with protocol **MV ELITe_PC**,

as a Negative amplification Control, use molecular grade water (not provided with this kit) in association with protocol **MV ELITe_NC**.

Note: The **ELITe InGenius** system requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification 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.
Furthermore the amplification controls must be re-run when:
- a new lot of amplification reagents is started,
- the results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

It is recommended to validate periodically the whole analysis procedure, extraction and amplification, by testing as process controls a negative tested sample and a positive tested sample or a reference material.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

PROCEDURE

The procedure to use the **Meningitis Viral ELITe MGB® Panel** 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** and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, MV Positive Control, MV Negative Control) are run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification controls approved or valid, run 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 **Meningitis Viral ELITe MGB® Panel** is described in the table below

Assay protocol for Meningitis Viral ELITe MGB® Panel			
Name	**Matrix**	**Report**	**Characteristics**
MV ELITe_CSF_200_100	cerebrospinal fluid (CSF)	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 15 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 **Meningitis Viral ELITe MGB® Panel** can be used with the **ELITe InGenius** system in order to perform:

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

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 "Location Information Server" (LIS) through which it is possible to send 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

Before starting the session it is important to do the following:

1. Remove and thaw at room temperature (+18 / 25 °C) the test tubes containing the samples to be analysed. Mix by vortexing for 10 seconds, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep on ice,
2. Remove and thaw for 30 minutes at room temperature (+18 / 25 °C) the MV primer and probe mix (VIOLET cap) test tubes needed for the session, remembering that the content of each test tube is enough for 48 reactions. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep on ice,
3. Remove and thaw for 30 minutes at room temperature (+18 / 25 °C) the MV buffer mix (ORANGE cap) test tubes necessary for the session, remembering that the content of each tube is sufficient to set up 48 reactions. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep on ice,
4. When needed, remove the MV enzyme (YELLOW cap) test tubes necessary for the session remembering that the content of each tube is sufficient to set up 48 reactions. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep on ice,

Note: The MV enzyme should not be exposed to temperatures above -20 °C for more than 10 minutes. After thawing it is recommended to store it on ice or in the cooling block

5. Prepare one 2 mL tube (not provided with the kit) for the complete reaction mixture MV PCR Mix and mark them in a recognizable manner with a permanent marker,
6. Calculate the volumes of the three components provided by kit that are needed for preparing the complete reaction mixture MV PCR Mix on the basis of the number of samples to be analyzed, as described in the following table.

Note: In order to calculate the volumes of the three components it is necessary to define the number of reactions (N) of the session by counting the number of the samples to be tested plus one reaction (when analyzing from 1 to 4 samples), two reactions (when analyzing from 5 to 8 samples), or three reactions (when analyzing from 9 to 12 samples) as safety margin.

Reaction Number	MV primer and probe mix	MV buffer mix	MV enzyme
1	1.5 µL	12.5 µL	1 µL
N	N x 1.5 µL	N x 12.5 µL	N x 1 µL

7. Prepare the complete reaction mixture MV PCR Mix by adding to the dedicated tube the calculated volumes of the three components.

Note: Prepare the complete reaction mixture immediately before loading it into the instrument.

Note: The complete reaction mixture **cannot** be stored, it is stable for 3 consecutive runs if loaded into the instrument (Inventory Area), but it's important to homogenize it between each run.

Note: Do not immerge the whole tip into the liquid when pipetting to avoid waste of material and to obtain accurate volumes; pipetting must be done very slowly to prevent air bubbles; wipe the tip against the edge of the vessel to remove excess liquid outside the tip before dispensing; take care to change the tips after each pipetting step.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

8. Mix by vortexing at low speed for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep on ice;
9. Thaw the **500 Internal Control** test tubes for the session. Each tube is sufficient for 32 extractions. Mix gently, spin down the content for 5 seconds before any session.

To setup an integrated run, carry out the following steps as per the GUI.

10. Select "Perform Run" from the "Home" screen.
11. Ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 100 µL.
12. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
13. Select the Assay protocol to be used in the "Assay" column (i.e. MV ELITe_CSF_200_100).
14. Ensure that the "Protocol" displayed is: "Extract + PCR".
15. 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 "Sonicator Tube".

    Click "Next" to continue the setup.
16. Load the 500 Internal Control test tube and the MV PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
17. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
18. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 15, following the GUI instruction. Click "Next" to continue the setup.
19. Close the instrument door.
20. Press "Start" to start the run.

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

Note: At the end of the run the remaining Extracted Sample 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 other consumables must be removed from the instrument and disposed without producing environmental contaminations. Avoid the spilling of the reaction products.

B. Amplification run

1. Remove and thaw at room temperature (+18 / 25 °C) the test tubes containing the extracted samples. Mix by vortexing for 10 seconds, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep on ice,
2. Prepare the complete reaction mixture MV PCR Mix in sufficient volume for the session, as described in paragraph A. Integrated run (from point 2 to 8; do not thaw the Internal Control).

   To set up the amplification run carry on the steps below following the GUI:
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
6. Select the Assay protocol to be used in the “Assay” column (i.e. MV ELITe_CSF_200_100).
7. Select “PCR Only” in the “Protocol” column.
8. Ensure the sample loading position in the “Sample Position” column is “ExtraTube (bottom row)”. Click “Next” to continue the setup.
9. Load MV PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR Cassettes” and the extracted Nucleic Acid samples 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, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample 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 other consumables must be removed from the instrument and disposed without producing environmental contaminations. Avoid the spilling of the reaction products.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF RTS507ING

C. Amplification run for Positive Control and Negative Control

1. Prepare the complete reaction mixture MV PCR Mix in sufficient volume for the session, as described in paragraph A. Integrated run (from point 2 to 8; do not thaw the 500 Internal Control).
2. Thaw the tube MV Positive Control, for Positive Control amplification. Each tube is sufficient for 6 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL the molecular biology grade water for the sessions in one Elution tube, provided with the ELITe InGenius SP 200 Consumable Set.

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

4. Select “Perform Run” from the “Home screen”.
5. In the Track of interest, select the Assay protocol to be used in the “Assay” column.
6. For the positive control, select MV ELITe_PC in the “Assay” column and fill in the lot number and expiry date of MV Positive Control,
7. For the negative control, select MV ELITe_NC and fill in the lot number and expiry date of the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load MV PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR Cassettes”, the MV-Positive Control tube and the negative control tube 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, store the results and to print and save the report.

Note: The results of Positive Control and Negative Control amplification runs are used by the instrument software to set up the “Control Charts”. Four Positive Control and Negative Control results, from four different runs, are requested to set up the control chart. After that, the results of Positive control and Negative Controls are used for monitoring the amplification step performances. Refer to the instrument user’s manual for more details.

Note: At the end of the run the remaining Positive Control must be removed from the instrument, capped, identified and stored at -20 °C. Avoid spilling the Positive Control. The remaining Negative Control must be disposed.

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

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 “Location Information Server” (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 **Meningitis Viral ELITe MGB® Panel** through the following procedure:

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

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of target genes (“HSV1”, “HSV2”, “VZV”) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocols “MV ELITe_PC” and “MV ELITe_NC”.

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

Before analysing any sample it is absolutely mandatory to verify that amplification Positive Control and Negative Control were run with the lot of amplification reagent to be used and results are approved and valid. The availability of “Approved” (Status) amplification Positive Control and Negative Control results is shown in the “Controls” window of the GUI. If the amplification Positive Control and Negative Control results are missing, generate them as described above.

Note: When the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the “not passed” message is shown on the “Controls” screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

Note: When 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 amplification of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the probes of target genes (“HSV1”, “HSV2”, “VZV”) and by the probe of Internal Control (“IC”) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocol MV ELITe _CSF_200_100.

Note: Before analysing any sample, verify that amplification controls were run with the lot of amplification reagent to be used and results are approved and valid. The availability of “Approved” (Status) amplification control results is shown in the “Controls” window of the GUI. If the amplification control results are missing, generate them as described above.

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**
MV Positive Control	APPROVED
2) Negative Control	**Status**
MV Negative Control	APPROVED

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

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 of a sample are listed the table below. The different genes are detected or not detected in combination.

Result of sample run	**Interpretation**
HSV1: DNA Detected.	**Herpes simplex virus 1 DNA was detected** in the sample.
HSV2: DNA Detected.	**Herpes simplex virus 2 DNA was detected** in the sample.
VZV: DNA Detected.	**Varicella zoster virus DNA was detected** in the sample.
HSV1: DNA Not Detected or below LoD.	**Herpes simplex virus 1 DNA was not detected** in the sample. The sample is negative for this gene or its concentration is below the Limit of Detection of the assay.
HSV2: DNA Not Detected or below LoD.	**Herpes simplex virus 2 DNA was not detected** in the sample. The sample is negative for this gene or its concentration is below the Limit of Detection of the assay.
VZV: DNA Not Detected or below LoD.	**Varicella zoster virus DNA was not detected** in the sample. The sample is negative for this gene or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor carry-over)

Samples not suitable for result interpretation are reported as “Invalid - Retest Sample” by the **ELITe InGenius software**. 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 and false negative.

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 suitable for analysis but in which it was not possible to detect herpes simplex virus 1 & 2 and varicella zoster virus DNA are reported as: “Not Detected or below LoD". In this case it cannot be excluded that the DNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

Note: The results obtained with this assay must be interpreted taking into consideration 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 by selected Track.

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

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The limit of detection (LoD) of this assay used in association to CSF samples and ELITe InGenius system was verified by testing serial dilutions of plasmids (spiked in negative CSF and then extracted and amplified to mimic real samples) containing target sequence for each pathogen of the multiplex assay (80,000-40,000-20,000-10,000-5,000-2,500-1,250-625-312 copies/ml).

The absolute LoD from the results of 10 replicates of the plasmid serial dilutions was defined as the last dilution step at which 100% of replicates are detected as positive.

The final results are reported in the following table.

Limit of Detection for CSF samples and ELITe InGenius System (copies / mL)	
Target	**LoD (copies / mL)**
herpes simplex virus 1 (HSV1)	2,500
herpes simplex virus 2 (HSV2)	1,250
varicella zoster virus (VZV)	1,250

The analytical sensitivity was also analysed by regression analysis. A linear regression was performed on plasmid dilution series, showing 100% of positivity rate, to calculate the regression coefficient R^2 and the slope. R2 values for all three pathogens were higher than 0.99 showing the good linearity of detection within this range of dilution.

Repeatability

The repeatability, as intra-run imprecision, of this assay in association with the ELITe InGenius system was tested by performing 10 replicates of two concentrations of a characterized clinical sample (10xLoD and 3xLoD) for each pathogen, tested through extraction and PCR process with the same operator, reagent lots, instrument and in the same environment.

The data analysis of the intra-assay shows a very good repeatability of the results with coefficient of variation lower than 1.5% for each pathogen's samples and for each concentration (10xLoD or 3xLoD).
A summary of results is shown below.

Repeatability of the Meningitis Viral ELITe MGB® Panel					
Sample	**Concentration**	**Ct Mean**	**σ**	**CV%**	**% positive**
HSV1_sample 1	10xLoD	30.2	0.3	1.0	100
HSV1_sample 1	3xLoD	32.1	0.4	1.1	100
HSV2_sample 2	10xLoD	31.3	0.3	1.0	100
HSV2_sample 2	3xLoD	33.9	0.3	0.9	100
VZV_sample 3	10xLoD	30.3	0.3	1.1	100
VZV_sample 3	3xLoD	31.7	0.3	1.0	100

Reproducibility

The Reproducibility, as "Batch to batch" and "Instrument to Instrument" variability, of this assay in association with the ELITe InGenius™ system was performed with the same samples and with the same reagent lots but with different operator, time, instrument and laboratory.

Precision was expressed on the basis of statistical measurements of imprecision, such as standard deviation (σ) and coefficient of variation (CV).

Analysis of the inter-assay shows a high reproducibility of the results with CV values lower than 1.5%.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF RTS507ING

A summary of results is shown below.

Reproducibility of the Meningitis Viral ELITe MGB® Panel					
Sample	**Concentration**	**Ct Mean**	**σ**	**CV%**	**% positive**
HSV1_sample 1	10xLoD	30.5	0.4	1.4	100
HSV1_sample 1	3xLoD	32.3	0.4	1.3	100
HSV2_sample 2	10xLoD	31.2	0.1	0.5	100
HSV2_sample 2	3xLoD	33.2	0.2	0.6	100
VZV_sample 3	10xLoD	29.7	0.3	0.9	100
VZV_sample 3	3xLoD	31.7	0.4	1.3	100

Analytical specificity and Reference Material testing

The specificity of this assay in association with the ELITe InGenius system was evaluated by performing an extraction and PCR process on QCMD panels (Qnostics Ltd, U.K.) of herpes simplex viruses (HSVDNA16C1-2) and varicella-zoster virus (VZVDNA16C1-2).

All the positive samples were detected with the **Meningitis Viral ELITe MGB® Panel.** However the sample VZVDNA16C1-02 was only detected on 1 over 2 replicates with a high Ct value (36.9). This sample was detected in 37.8% on the total number of datasets reported for this panel suggesting that this is a very low positive sample.

None of the negative samples of the HSVDNA16C1-2 or VZVDNA16C1-2 QCMD panels were detected as positive with the **Meningitis Viral ELITe MGB® Panel**. Moreover, the non-specific targets for the used QCMD panels (VZV for HSVDNA16C1-2 panel or HSV1 and HSV2 for the VZVDNA16C1-2 panel) were all negative on the ELITe InGenius™ showing the good specificity of the assay.

A summary of results is shown below.

Sample	Description	Sample status	Ct value		
			HSV1	HSV2	VZV
HSVDNA16C1-01	Herpes Simplex Virus 2 (09-015681)	frequently detected	neg	28.7	neg
HSVDNA16C1-02	Herpes Simplex Virus 1 (95/1906)	frequently detected	31.1	neg	neg
HSVDNA16C1-03	HSV Negative	negative	neg	neg	neg
HSVDNA16C1-04	Herpes Simplex Virus 1 (MacIntyre)	frequently detected	31.3	neg	neg
HSVDNA16C1-05	Herpes Simplex Virus 2 (09-015681)	detected	neg	33.9	neg
HSVDNA16C2-01	Herpes Simplex Virus 1 (95/1906)	detected	33.9	neg	neg
HSVDNA16C2-02	Herpes Simplex Virus 2 (09-015681)	frequently detected	neg	30.1	neg
HSVDNA16C2-03	Herpes Simplex Virus 1 (95/1906)	frequently detected	31.2	neg	neg
HSVDNA16C2-04	HSV Negative	negative	neg	neg	neg
HSVDNA16C2-05	Herpes Simplex Virus 2 (MS)	detected	neg	34.0	neg
VZVDNA16C1-01	VZV Negative	negative	neg	neg	neg
VZVDNA16C1-02	Varicella-zoster virus (Ellen)	infrequently detected	neg	neg	36.9 (1/2)
VZVDNA16C1-03	Varicella-zoster virus (63/1444)	frequently detected	neg	neg	32.9
VZVDNA16C1-04	Varicella-zoster virus (Ellen)	frequently detected	neg	neg	31.2
VZVDNA16C1-05	Varicella-zoster virus (9/84)	frequently detected	neg	neg	29.5
VZVDNA16C2-01	Varicella-zoster virus (OKA)	frequently detected	neg	neg	32.1
VZVDNA16C2-02	Varicella-zoster virus (9/84)	frequently detected	neg	neg	29.6
VZVDNA16C2-03	Varicella-zoster virus (9/84)	frequently detected	neg	neg	33.3
VZVDNA16C2-04	Varicella-zoster virus (Ellen)	frequently detected	neg	neg	31.1
VZVDNA16C2-05	Varicella-zoster virus (Ellen)	detected	neg	neg	33.8

The specificity was also evaluated testing about 80 clinical samples containing bacteria, parasites and viruses. No other pathogens than expected were detected.

A summary of results is shown below.

Pathogen (viruses)	Result
Adenovirus	negative
Astrovirus	negative
Coronavirus 229	negative
Coronavirus 43	negative
Coronavirus 63	negative
Cytomegalovirus	negative
Enterovirus	negative
Epstein-Barr virus	negative
Herpes simplex virus 1	**positive**
Herpes simplex virus 2	**positive**
Human herpes virus 6	negative
Human herpes virus 7	negative
Human metapneumovirus	negative
Influenza A virus	negative
Influenza B virus	negative
Measles virus	negative
Mumps virus	negative
Norovirus G1	negative
Norovirus G2	negative
Parainfluenza 1	negative
Parainfluenza 2	negative
Parainfluenza 3	negative
Parainfluenza 4	negative
Parvovirus B19	negative
Polyomavirus 1 (BKV)	negative
Respiratory syncytial virus A	negative
Respiratory syncytial virus B	negative
Rhinovirus	negative
Rotavirus	negative
Sapovirus	negative
Varicella-zoster virus	**positive**

Pathogen (bacteria and parasites)	Result	Pathogen (bacteria and parasites)	Result
Aeromonas hydrophilia	negative	*Legionella pneumophila*	negative
Bacillus ssp.	negative	*Legionella ansia*	negative
Bifidobacterium	negative	*Listeria monocytogenes*	negative
Bordetella pertussis	negative	*Moraxella catarrhalis*	negative
Camplyobacter jejuni	negative	*Morganella morganii*	negative
Campylobacter coli	negative	*Mycoplasma genitalium*	negative
Chlamydia trachomatis	negative	*Mycoplasma pneumoniae*	negative
Citrobacter freundii	negative	*Neisseria gonorrhoeae*	negative
Clostridium difficile	negative	*Proteus mirabilis*	negative
Clostridium perfringens	negative	*Proteus vulgaris*	negative
EHEC vtx+	negative	*Rhodococcus equi*	negative
EIEC	negative	*Salmonella typhimurium*	negative
Enterococcus faecalis	negative	*Shigella boydii*	negative
EPEC	negative	*Staphylococcus aureus*	negative
ETEC	negative	*Streptococcus pneumoniae*	negative
Haemophilus influenzae	negative	*Treponema pallidum*	negative
Hafnia alvei	negative	*Vibrio cholerae*	negative
Klebsiella oxytoca	negative	*Yersinia enterocolitica*	negative
Klebsiella pneumoniae	negative		

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF RTS507ING

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of this assay, as confirmation of positive clinical samples, was evaluated by testing a set of archived specimens obtained from different positive patients, previously characterized by reference method. In addition a set of contrived samples were created diluting the targets DNA from reference material into different negative donor samples

A summary of the results after discrepant analysis is reported in the table below.

Sample	N	Positive	Negative	Invalid
HSV1 positive CSF	10	9	1	0
HSV1 spiked CSF	22	22	0	0
HSV2 positive CSF	10	10	0	0
HSV2 spiked CSF	20	20	0	0
VZV positive CSF	10	10	0	0
VZV spiked CSF	20	20	0	0

The discrepant sample, that had a Ct close to the limit of detection of the reference method, could be explained by the very low concentration of viruses in the samples, below the limit of detection of the method; such samples could stochastically result either positive or negative.

In this test the diagnostic sensitivity was equal to 96.9% for HSV1 and 100% for both HSV2 and VZV

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated by testing a set of archived specimens from different negative donors previously characterized by reference method.

A summary of the results after discrepant analysis is reported in the table below.

Sample	N	Positive	Negative	Invalid
HSV1, HSV2 and VZV negative CSF	30	0	30	0

In this test the specificity was equal to 100% for HSV1, HSV2 and VZV

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "Meningitis Viral ELITe MGB Panel", FTP RTS507ING.

PROCEDURE LIMITATIONS

Use this product only with DNA extracted from the following clinical samples: CSF

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

The results obtained with this product depend on an adequate identification, collection, transport storage and processing of the samples. 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 must be handled by qualified personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid incorrect results.

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.

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.

In case of co-infections, the sensitivity of one target can be affected by the amplification of a second target.

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 the final results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target 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, as the emergency diagnosis, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting 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**
Instrument setting 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**
Instrument setting error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
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 with a 1 : 2 dilution in in molecular biology grade water of the sample in a “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
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.
	Keep away from sunlight.
	Manufacturer.

Meningitis Viral ELITe MGB® Panel
reagent for DNA Real Time amplification

REF **RTS507ING**

NOTICE TO PURCHASER: LIMITED LICENSE

TaqMan™ MGB® detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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