Instructions for use

HPV PLUS ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

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IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yyyy)
00	new product development	20/09/2024

TABLE OF CONTENT

1 INTENDED USE

The product **HPV PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the **genomic DNA of Human Papilloma Virus** 14 high-risk types as follow: detection and typing of the high-risk HPV 16, 18, 31, 45, detection of high-risk HPV HR1 group (33, 52, 58) and detection of high-risk HPV HR2 group, non-vaccinal types, (35, 39, 51, 56, 59, 66 and 68), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human cervical specimens placed in alcohol-based fixative for cytology.

The product is intended for use as an aid in the diagnosis of HPV infections, in patients with cervical cytology or HPV molecular positive test results. The results must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting HPV DNA isolated from specimens and amplified using the assay reagent **HPV PLUS PCR Mix** that contains primers and probes with ELITe MGB and TaqMan™ MGB technology.

The probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold Cycles (Ct) and the melting temperatures (Tm).

In the probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore.

Note that, for ELITe MGB probes, the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

The **HPV PLUS ELITe MGB Kit** provides the assay reagent **HPV PLUS PCR Mix**, an optimized and stabilized PCR Mixture that contains the specific primers and probes for:

- HPV16 and HPV31 **E6/E7** genomic region, detected in Channel 1 (**HPV16_31**) and discriminated by melting temperature. The probes are stabilized by MGB, quenched by Eclipse Dark Quencher® and labelled by FAM dye,
- HPV18 and HPV45 **E6/E7** genomic region, detected in Channel 6 (**HPV18_45**) and discriminated by melting temperature. The probes are stabilized by MGB, quenched by Eclipse Dark Quencher and labelled by AquaPhluor® 690 (AP690) dye,
- HPV HR1 (HPV33, HPV52, HPV58) **E6/E7** genomic region, detected in Channel 5 (**HPV_HR1**). The probes are stabilized by MGB, quenched by Eclipse Dark Quencher and labelled by AquaPhluor 639 (AP639) dye,
- HPV HR2 (HPV35, HPV39, HPV51, HPV56, HPV59, HPV66, HPV68) **E6/E7** genomic region, detected in Channel 4 (**HPV_HR2**). The probes are stabilized by MGB, quenched by Eclipse Dark Quencher and labelled by AquaPhluor 593 (AP593) dye,
- Endogenous Internal Control, specific for the human **beta globin** gene, detected in Channel 2 (**IC**). The probe is stabilized by MGB, quenched by Eclipse Dark Quencher and labelled by AquaPhluor 525 (AP525) dye.

The **HPV PLUS PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase

The endogenous Internal Control monitors for the cellularity of sample, extraction and PCR efficiency.

The **HPV PLUS ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius (12 tests each tube)**, with 20 µL used per reaction.

The **HPV PLUS ELITe MGB Kit** can be also used in association with equivalent instruments.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
HPV PLUS PCR Mix ref. RTS402ING	Mixture of reagents for Real-Time PCR in tube with **WHITE cap**	**8 x 280 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.

- Disposable nitrile powder-free gloves or similar material.

- Vortex mixer.

- Bench centrifuge (~5,000 RPM).

- Bench microcentrifuge (~13,000 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).

- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).

- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the amplification positive and negative controls and the consumables are **not** provided with this product.

For automated extraction of nucleic acids, Real-Time PCR and result interpretation of samples, the following products are required.

Table 2

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S. p. A., EG SpA, ref. INT030) **ELITe InGenius Software** version 1.3.0.19 (or later) **HPV PLUS ELITe _PC**, Assay Protocol with parameters for Positive Control analysis **HPV PLUS ELITe _NC**, Assay Protocol with parameters for Negative Control analysis **HPV PLUS ELITe_Cyt_Sense_200_100**, Assay Protocol with parameters for cervical specimens analysis.	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR), **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000) **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only **HPV PLUS- ELITe Positive Control** (EG SpA, ref. CTR402ING) **ThinPrep® Pap Test PreservCyt® Solution** (Hologic, Inc., code 70098-002, 20 mL of PreservCyt® Solution). **BD SurePath™ Collection Vial 10 mL** (Becton, Dickinson and Company, codes 491439 / 491438 / 491440, 10 mL of SurePath® Preservative Fluid).
ELITe BeGenius (EG SpA, ref. INT040) **ELITe BeGenius Software** version 2.2.1 (or later) **HPV PLUS ELITe_Be _PC**, Assay Protocol with parameters for Positive Control analysis **HPV PLUS ELITe_Be _NC**, Assay Protocol with parameters for Negative Control analysis **HPV PLUS ELITe _Be _Cyt_Sense_200_100**, Assay Protocol with parameters for cervical specimens analysis.	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) 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 infectious. 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 neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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 before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use 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.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR products.

Laboratory 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 reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this 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 extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent contamination.

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
HPV PLUS PCR Mix	-20°C or below (protected from light)	one month	up to seven	up to seven separate* sessions of about three hours each or up to 7 consecutive hours (2 sessions of about 3 hours each and the time needed to start a third session)

*with intermediate freezing

8 SPECIMENS AND CONTROLS

8.1 Specimens

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions:

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Cervical specimen for liquid cytology	ThinPrep® Pap Test PreservCyt® Solution (Hologic, Inc.)	≤ 6 months	≤ 6 months	NR	NR
	BD SurePath™ Collection Vial 10 mL (Becton, Dickinson and C.)	≤ 3 months	≤ 6 months	NR	NR

NR: not recommended.

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius or ELITe BeGenius** with the indicated matrices.

Table 5

Assay Protocols for HPV PLUS ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Cervical specimen for liquid cytology	**ELITe InGenius**	**HPV PLUS ELITe_Cyt_Sense_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**HPV PLUS ELITe_Be_Cyt_Sense_200_100**	Positive / Negative	

For all protocols, 200 µL of sample must be transferred into Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the “7 WARNINGS AND PRECAUTIONS page 6” section.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to “Potentially Interfering Substances” in the 11 PERFORMANCE CHARACTERISTICS page 17 section to check data concerning interfering substances.

8.2 PCR controls

PCR control results must be generated and approved for each lot of PCR reagent.

- For the Positive Control, use the product **HPV PLUS - ELITe Positive Control** (not provided with this kit) with the **HPV PLUS ELITe _PC** or **HPV PLUS ELITe _Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **HPV PLUS ELITe _NC** or **HPV PLUS ELITe _Be_NC** Assay Protocols.

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent. PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and Negative Controls.The PCR controls must be re-run if any of the following events occur:

- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** or **ELITe BeGenius**.

8.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

9 ELITe InGenius PROCEDURE

The procedure to use the **HPV PLUS ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 6

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only),
		C) Positive Control and Negative Control run (PCR Only).
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

9.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in “**CLOSED**” mode,
- in the “Controls” menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 EG SpA (see “Specimens and Controls”)

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

9.2 STEP 2 - Session Setup

The **HPV PLUS ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **12 tests** in optimized conditions (2 or more tests per session).Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI

Table 7

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. For this assay, 200 µL of sample must be transferred in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. (Each tube is sufficient for 4 reactions.) **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with ELITe InGenius SP 200 Consumable Set.
2	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.
3	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
4	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	Not applicable

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
5	**Select the Assay Protocol** in the "Assay" column (see "8 SPECIMENS AND CONTROLS page 7").	**Select the Assay Protocol** in the "Assay" column (see "8 SPECIMENS AND CONTROLS page 7").	**Select the Assay Protocol** in the "Assay" column (see "8 SPECIMENS AND CONTROLS page 7"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
6	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
7	Select the sample loading position as "Extraction Tube" in the "Sample Position" column.	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
8	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
9	**Load PCR Mix** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
11	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
12	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
13	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tubes with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
14	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
15	Close the instrument door.	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".	Press "Start".

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results, 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, identified and stored at -20 ±10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the **Positive Control** .The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”).Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **HPV PLUS ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,
2. Validation of sample results,
3. Sample result reporting.

9.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius Software** interprets the PCR results for the targets of the Positive Control and Negative Control reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct and Tm values are used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls).They can be viewed and approved by “Administrator” or “Analyst” users, following the GUI instructions.

The Positive Control and Negative Control results expire after **15 days**.

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.2 B. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channels **HPV16-31, HPV18-45, HPV HR1** and **HPV HR2**) and the Internal Control (channel **IC**) with the**HPV PLUS ELITe_Cyt_Sense_200_100** Assay Protocol parameters.

Results are shown in “Results Display” screen.

The sample results can be approved when the two conditions in the table below are true.

Table 8

1) Positive Control	**Status**
HPV PLUS Positive Control	APPROVED
2) Negative Control	**Status**
HPV PLUS Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using 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.

Table 9

Result of sample run	Interpretation
HPV16_31:DNA Detected Type HPV16	**HPV16-31 DNA was detected** in the sample. The type is **HPV16**.
HPV16_31:DNA Detected Type HPV31	**HPV16-31 DNA was detected** in the sample. The type is **HPV31**.
HPV16_31:DNA Detected Typing not determined	**HPV16-31 DNA was detected** in the sample but the analysis for HPV16-31 typing was not feasible.
HPV18_45:DNA Detected Type HPV18	**HPV18-45 DNA was detected** in the sample. The type is **HPV18**.
HPV18_45:DNA Detected Type HPV45	**HPV18-45 DNA was detected** in the sample. The type is **HPV45**.
HPV18_45:DNA Detected Typing not determined	**HPV18-45 DNA was detected** in the sample but the analysis for HPV18-45 typing was not feasible.
HPV_HR1:DNA Detected	**HPV HR1** (HPV33, HPV52 or HPV58) **DNA was detected** in the sample.
HPV_HR2:DNA Detected	**HPV HR2** (HPV35, HPV39, HPV51, HPV56, HPV59, HPV66 or HPV68) **DNA was detected** in the sample.
HPV16_31:DNA Not detected or below the LoD	**HPV16-31 DNA was not detected** in the sample. The sample is negative for HPV16 and HPV31 DNA, or its concentration is below the Limit of Detection of the assay.
HPV18_45:DNA Not detected or below the LoD	HPV18-45 DNA was not detected in the sample. The sample is negative for HPV18 and HPV45 DNA, or its concentration is below the Limit of Detection of the assay.
HPV-HR1:DNA Not detected or below the LoD	HPV HR1 DNA was not detected in the sample. The sample is negative for HPV33, HPV52 and HPV58 DNA, or its concentration is below the Limit of Detection of the assay.
HPV-HR2:DNA Not detected or below the LoD	HPV HR2 DNA was not detected in the sample. The sample is negative for HPV35, HPV39, HPV51, HPV56, HPV59, HPV66 and HPV68 DNA, or its concentration is below the Limit of Detection of the assay.
Invalid-Retest Sample	**Not a valid assay result** caused by Internal Control failure (due to e. g., incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid - Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g., incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode. (see 14 TROUBLESHOOTING page 33).

Samples reported as “HPV16-31:DNA Not detected or below LoD", “HPV18-45:DNA Not detected or below LoD", “HPV_HR1: DNA Not detected or below LoD", “HPV_HR2: DNA Not detected or below LoD", are suitable for analysis but HPV DNA was not detected. In this case, the sample may be either negative for HPV DNA or HPV DNA is present at a concentration below the Limit of Detection of the assay (see 11 PERFORMANCE CHARACTERISTICS page 17).

Samples reported as “HPV16_31:DNA Detected Typing not determined” and “HPV18_45:DNA Detected Typing not determined” are suitable for analysis and HPV DNA was detected in the sample, but the analysis for typing was not feasible. In this case, the sample is positive for one or both targets in that channel.

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

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

9.3.3 Sample result reporting

- The sample results are stored in the database and reports can be exported as “Sample Report” and “Track Report”.
- The “Sample Report” shows the results details by selected sample (SID).
- The “Track Report” shows the results details by selected Track.
- The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

10 ELITe BeGenius PROCEDURE

The procedure to use the **HPV PLUS ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 10

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only),
		C) Positive Control and Negative Control run (PCR Only).
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in “**CLOSED**” mode,

- in the “Controls” menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 EG SpA (see “Specimens and Controls”).

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

10.2 STEP 2 - Session Setup

The **HPV PLUS ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for 12 tests in optimized conditions (2 or more tests per session).Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 11

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature). For this assay, **200 µL of sample** must be transferred in a 2 mL Sarstedt tube previously labelled.	**Thaw the Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block. Prepare the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
2	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen	Select “**Perform Run**” from the “Home” screen.
3	Remove all the Racks from the “Cooler Unit” and place them on the preparation table.	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) of the “Cooler Unit” and place them on the preparation table	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) from the “Cooler Unit” and place them on the preparation table.
4	Select the “Run mode”: “**Extract + PCR”.**	Select the “Run mode”: “**PCR Only**”.	Select the “Run mode”: “**PCR Only**”.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
5	**Load the samples** into the “Sample Rack”. When secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”.	**Load the samples** into the “Elution Rack”.	**Load the Positive Control and Negative Control** tubes into the “Elution Rack”.
6	**Insert** the “**Sample Rack**” into the “Cooler Unit” starting from the “Lane 5” (L5). If needed, insert the “Sample ID” (SID) for each “Position” used (If secondary tubes are loaded, flag “2 mL Tube”. If secondary tubes are not barcoded, type manually the “Sample ID”).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from “Lane 3” (L3). If needed, for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from the “Lane 3” (L3). If needed, for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
7	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
8	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL.
9	Select the Assay Protocol in the “Assay” column (see “8 SPECIMENS AND CONTROLS page 7”).	Select the Assay Protocol in the “Assay” column (see “8 SPECIMENS AND CONTROLS page 7”).	Select the Assay Protocol in the “Assay” column (see “8 SPECIMENS AND CONTROLS page 7”).
10	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
	NOTE When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
11	Load the “Elution tubes” into the “Elution Rack” (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
12	Insert the “Elution Rack” into the “Cooler Unit” starting from “Lane 3” (L3). When more than 12 samples are processed, repeat using “Lane 2” (L2).	Not applicable	Not applicable
13	Click “Next” to continue.	Not applicable	Not applicable
14	**Load PCR Mix** into the “Reagent/Elution Rack”.	**Load the PCR Mix** into “Reagent/Elution Rack”.	**Load the PCR Mix** into “Reagent/Elution Rack”.
15	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
16	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
17	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
18	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
19	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the **"PCR Rack**" with "PCR Cassette" in the Inventory Area.
20	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
21	Load the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables.	Not applicable	Not applicable
22	Close the instrument door.	Close the instrument door.	Close the instrument door.
23	Press "Start".	Press "Start".	Press "Start".

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results, 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, identified and stored at -20 ±10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **HPV PLUS ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,
2. Validation of sample results,
3. Sample result reporting.

NOTE

Please, refer to the same paragraph of the **ELITe InGenius** Procedure for the details.

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined for ELITe BeGenius instrument, testing a pool of negative samples of cervical specimen collected in ThinPrep spiked by SiHa cells (HPV16) and HeLa cells (HPV18) reference materials together.

Probit regression analysis was performed on the results and the LoD was estimated as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Table 12 Limit of Detection for Cervical samples collected in ThinPrep on ELITe BeGenius

Cell line	LoD (cells / mL)	95% confidence interval limits	
		Lower limit	Upper limit
SiHa (HPV16)	202	142	368
HeLa (HPV18)	56	40	98

The calculated LoD value was verified by testing on ELITe BeGenius and ELITe InGenius instruments negative pools of cervical specimen collected in ThinPrep and in SurePath spiked with WHO International Standards of HPV16, HPV18, HPV31, HPV33 and HPV45 genotypes and with plasmid DNA of HPV35 genotype at a titre corresponding to the calculated HeLa cell LoD value.

The results of LoD confirmation are reported in the following table.

Table 13 LoD Confirmation with cervical samples collected in ThinPrep and SurePath on ELITe BeGenius and ELITe InGenius

Sample	LoD Confirmation					
	HPV16	HPV18	HPV31	HPV33	HPV45	HPV35
target titer IU / mL (or *copies / mL)	1,700	1,700	1,700	2,000	1,700	1,700*
Replicates (N)	24	24	24	24	24	24
% rate of positive results	>87%	>87%	>87%	>87%	>87%	>87%

11.2 Inclusivity: Efficiency of detection for different high-risk HPV genotypes

The Inclusivity of the assay, as efficiency of detection for the main high-risk HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) was evaluated by *in silico* analysis. The analysis showed sequence conservation and absence of significant mutations. So, an efficient detection for the different strains or isolates is expected.

The Inclusivity was also verified through the analysis of the 14 main high-risk HPV genotypes using certified reference materials form different providers (ATCC, Vircell, Seracare, Acrometrix, NIBSC and Microbix) or plasmid DNAs (EG SpA) when no reference material was available.

The results are reported in the following table.

Table 14 Inclusivity test results on ELITe BeGenius

Target	Panel	Pos. / Rep.	Outcome
HPV16	WHO IU, NIBSC	6 / 6	HPV16_31:DNA Detected Type HPV16
	Acrometrix	6 / 6	HPV16_31:DNA Detected Type HPV16
	Microbix	6 / 6	HPV16_31:DNA Detected Type HPV16
	ATCC	6 / 6	HPV16_31:DNA Detected Type HPV16
	SeraCare	6 / 6	HPV16_31:DNA Detected Type HPV16
	Vircell	6 / 6	HPV16_31:DNA Detected Type HPV16
HPV18	WHO IU, NIBSC	6 / 6	HPV18_45:DNA Detected Type HPV18
	Acrometrix	6 / 6	HPV18_45:DNA Detected Type HPV18
	Microbix	6 / 6	HPV18_45:DNA Detected Type HPV18
	ATCC	6 / 6	HPV18_45:DNA Detected Type HPV18
	SeraCare	6 / 6	HPV18_45:DNA Detected Type HPV18
	Vircell	6 / 6	HPV18_45:DNA Detected Type HPV18
HPV31	WHO IU, NIBSC	6 / 6	HPV16_31:DNA Detected Type HPV31
	Microbix	6 / 6	HPV16_31:DNA Detected Type HPV31
	ATCC	6 / 6	HPV16_31:DNA Detected Type HPV31
HPV45	WHO IU, NIBSC	6 / 6	HPV18_45:DNA Detected Type HPV45
	Microbix	6 / 6	HPV18_45:DNA Detected Type HPV45

Table 14 Inclusivity test results on ELITe BeGenius (continued)

Target	Panel	Pos. / Rep.	Outcome
HPV33	WHO IU, NIBSC	6 / 6	HPV_HR1:DNA Detected
	Microbix	6 / 6	HPV_HR1:DNA Detected
HPV52	WHO IU, NIBSC	6 / 6	HPV_HR1:DNA Detected
HPV58	WHO IU, NIBSC	6 / 6	HPV_HR2:DNA Detected
HPV68	Acrometrix	6 / 6	HPV_HR2:DNA Detected
HPV39	Microbix	6 / 6	HPV_HR2:DNA Detected
HPV51	SeraCare	6 / 6	HPV_HR2:DNA Detected
HPV35	plasmid DNA	6 / 6	HPV_HR2:DNA Detected
HPV56	plasmid DNA	6 / 6	HPV_HR2:DNA Detected
HPV59	plasmid DNA	6 / 6	HPV_HR2:DNA Detected
HPV66	plasmid DNA	6 / 6	HPV_HR2:DNA Detected
Negative	Acrometrix	0 / 6	All targets:DNA Not detected
	Microbix	0 / 6	All targets:DNA Not detected
	SeraCare	0 / 6	All targets:DNA Not detected

All samples were correctly detected by the HPV PLUS ELITe MGB Kit

11.3 Co-infections

The potential interference among targets of the assay in case of co-infection was evaluated by a test of co-amplification of two targets of interest in the samples using HPV16, HPV18, HPV31, HPV45, HPV33 and HPV35 plasmid DNAs.

For each target, the lower concentration detectable in all replicates (5 / 5) is reported in the following table.

Table 15 Summary about test of co-infections on ELITe BeGenius

Target at high concentration	Target at low concentration
HPV16, 100,000 copies / reaction	HPV18, 1,000 copies /reaction
	HPV31, 10,000 copies /reaction
	HPV33, 3,000 copies /reaction
	HPV35, 2,000 copies /reaction
	HPV45, 500 copies /reaction
HPV31, 100,000 copies / reaction	HPV16, 5,000 copies /reaction
	HPV18, 1,000 copies /reaction
	HPV33, 4,000 copies /reaction
	HPV35, 500 copies /reaction
	HPV45, 1,000 copies /reaction

Table 15 Summary about test of co-infections on ELITe BeGenius (continued)

Target at high concentration	Target at low concentration
HPV18, 100,000 copies / reaction	HPV16, 750 copies /reaction
	HPV31, 500 copies /reaction
	HPV33, 3,000 copies /reaction
	HPV35, 500 copies /reaction
	HPV45, 15,000 copies /reaction
HPV45, 100,000 copies / reaction	HPV16, 750 copies /reaction
	HPV18, 5,000 copies /reaction
	HPV31, 500 copies /reaction
	HPV33, 5,000 copies /reaction
	HPV35, 500 copies /reaction
HPV33, 100,000 copies / reaction	HPV16, 1,000 copies /reaction
	HPV18, 1,000 copies /reaction
	HPV31, 500 copies /reaction
	HPV35, 500 copies /reaction
	HPV45, 1,000 copies /reaction
HPV35, 100,000 copies / reaction	HPV16, 2,000 copies /reaction
	HPV18, 500 copies /reaction
	HPV31, 500 copies /reaction
	HPV33, 4,000 copies /reaction
	HPV45, 1,000 copies /reaction

The HPV PLUS ELITe MGB Kit shows a minimal interference among targets in case of co-infection.

11.4 Potentially interfering organisms: Cross-reactivity

The potential cross-reactivity of unintended organisms and of other HPV genotypes not included in the Intended Use of the product that may be found in clinical specimen was evaluated for the assay by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, protozoa and fungi) and with the majority of HPV genotypes non included in the Intended Use.

The absence of cross-reactivity was also verified through the analysis of a panel of certified reference materials of unintended organisms (ATCC, Vircell, DSMZ, NIBSC) and of reference materials (NIBSC) and plasmid DNAs (EG SpA) containing the E6/E7 genes region of HPV genotypes non included in the Intended Use of the product (at about 10^5 copies / reaction).

The results are reported in the following table.

Table 16 Cross-reactivity test results on ELITe BeGenius with potentially interfering organisms

Sample	Positive / Replicates					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
Herpes simplex virus 1	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Herpes simplex virus 2	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Mycoplasma hominis	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Ureaplasma urealyticum	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Ureaplasma parvum	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Treponema pallidum	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Gardnerella vaginalis	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Mobiluncus mulieri	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Bacteroides fragilis	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Peptostreptococcus anaerobius	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Candida albicans	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Lactobacillus acidophilus	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Chlamydia trachomatis	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Neisseria gonorrhoeae	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Mycoplasma genitalium	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Trichomonas vaginalis	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV6	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV11	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV42	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV43	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV44	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV26	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV30	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV34	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV40	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV53	0 / 5	5 / 5	0 / 5	0 / 5	5 / 5	Cross-reactivity
HPV54	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV61	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV67	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV69	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity

Table 16 Cross-reactivity test results on ELITe BeGenius with potentially interfering organisms (continued)

Sample	Positive / Replicates					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
HPV70	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV72_a	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV72_b	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV73	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
HPV82	0 / 5	5 / 5	0 / 5	0 / 5	5 / 5	Cross-reactivity

Cross-reactivity was observed with HPV53 and HPV82 genotypes for HPV HR2 target using the HPV PLUS ELITe MGB Kit.

HPV82 genotype was detected as HPV HR2 target with high efficiency (≥ 1 x 10^3 copies / mL).

HPV53 genotype was detected as HPV HR2 target with low efficiency (≥ 1 x 10^6 copies / mL).

Both genotypes are classified as possibly carcinogenic (Group 2B, IARC Classification) on the basis of epidemiological, phylogenetic, and functional studies.

11.5 Potentially interfering organisms: Inhibition

The potential inhibition of unintended organisms and of other HPV genotypes not included in the Intended Use of the product that may be found in clinical specimen was evaluated for the assay through the analysis of a panel of certified reference materials of unintended organisms (ATCC, Vircell, DSMZ, NIBSC) and of reference materials (NIBSC) and plasmid DNAs (EG SpA) containing the E6/E7 genes region of HPV genotypes not included in the Intended Use (at about 10^5 copies / reaction), spiked with HPV PLUS - ELITe Positive Control at 1:10 dilution (100 copies / reaction of HPV16, HPV18, HPV33, HPV35 plasmid DNAs).

The results are reported in the following table.

Table 17 Inhibition test results on ELITe BeGenius with potentially interfering organisms

Sample spiked with PC 1:10	Positive / Replicates					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
Herpes simplex virus 1	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Herpes simplex virus 2	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Mycoplasma hominis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Ureaplasma urealyticum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Ureaplasma parvum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Treponema pallidum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Gardnerella vaginalis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Mobiluncus mulieri	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Bacteroides fragilis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Peptostreptococcus anaerobius	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition

Table 17 Inhibition test results on ELITe BeGenius with potentially interfering organisms (continued)

Sample spiked with PC 1:10	Positive / Replicates					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
Candida albicans	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Lactobacillus acidophilus	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Chlamydia trachomatis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Neisseria gonorrhoeae	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Mycoplasma genitalium	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Trichomonas vaginalis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV6	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV11	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV42	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV43	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV44	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV26	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV30	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV34	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV40	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV53	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV54	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV61	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV67	3 / 5	5 / 5	0 / 5	5 / 5	5 / 5	Inhibition
HPV69	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV70	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV72_a	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV72_b	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV73	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
HPV82	0 / 5	5 / 5	0 / 5	0 / 5	5 / 5	Inhibition

Inhibition was observed with HPV67 and HPV82 genotypes using the HPV PLUS ELITe MGB Kit.

The lowest detectable concentration of targets in the presence of these two HPV genotypes was defined, as reported in the following table.

Table 18

HPV genotype	Target under test	Concentration Target under test	Pos. / Rep.	Outcome
HPV67 (100,000 copies / rxn)	HPV16	200 copies / rxn	5 / 5	Slight inhibition
	HPV18	100 copies / rxn	5 / 5	No inhibition
	HPV33	1,000 copies / rxn	5 / 5	Partial inhibition
	HPV35	100 copies / rxn	5 / 5	No inhibition
HPV82 (100,000 copies / rxn)	HPV16	1,000 copies / rxn	5 / 5	Partial inhibition
	HPV18	1,000 copies / rxn	5 / 5	Partial inhibition
	HPV33	4,000 copies / rxn	5 / 5	Partial inhibition

Note: HPV82 is amplified as HPV-HR2 target, therefore a partial inhibition of amplification of other targets at low concentration is expected.

Both genotypes are classified as possibly carcinogenic (Group 2B, IARC Classification) on the basis of epidemiological, phylogenetic, and functional studies.

11.6 Potentially interfering substances: Cross-reactivity

The cross-reactivity by potentially interfering substances (endogenous and exogenous) that might be found in clinical specimen was evaluated for the assay by analysis of a panel of substances at relevant concentration in a pool of negative cervical specimen collected in ThinPrep.

The results are reported in the following table.

Table 19 Cross-reactivity test results on ELITe BeGenius with potentially interfering substances

Sample	Pos / Rep					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
Mucin	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Peripheral Blood Leucocytes (PBLs)	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Whole Blood	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Acyclovir	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Clotrimazole	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Metronidazole	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Nonoxinol 9	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Progesterone	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Vaseline	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Vagisil Deodorant	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity
Vagisil gel	0 / 5	0 / 5	0 / 5	0 / 5	5 / 5	No cross-reactivity

The test showed that all the tested substances do not cross-react with the targets using the HPV PLUS ELITe MGB Kit.

11.7 Potentially interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical samples was evaluated for the assay by analysis of a panel of substances at relevant concentration in a pool of negative cervical specimen collected in ThinPrep, spiked by reference materials (SiHa HPV16 cells, HeLa HPV18 cells, pHPV33 plasmid DNA, pHPV35 plasmid DNA) at 3x LoD.

The results are reported in the following table.

Table 20 Inhibition test results on ELITe BeGenius with potentially interfering substances

Sample spiked with reference materials	Pos / Rep					Outcome
	HPV 16-31	HPV HR2	HPV HR1	HPV 18-45	IC	
Mucin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Peripheral Blood Leucocytes (PBLs)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Whole Blood	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Acyclovir	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Clotrimazole	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Metronidazole	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Nonoxinol 9	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Progesterone	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Vaseline	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Vagisil Deodorant	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition
Vagisil gel 1%	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No inhibition

The test showed that all the tested substances do not interfere with target amplification using the HPV PLUS ELITe MGB Kit

Note: Inhibition was observed with Vaginal Moisturizer (Vagisil gel) at high concentration (> 1% w/v).

11.8 Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a pool of negative cervical specimen collected in ThinPrep as is or spiked by reference materials (SiHa HPV16 cells, HeLa HPV18 cells, pHPV33 plasmid DNA and pHPV35 plasmid DNA).

An example of Intra-Session Repeatability (on one day) Ct results is shown in the table below.

Table 21 Intra-Session Repeatability test - Ct results (one day / one lot)

Sample	Target	N	ELITe BeGenius				ELITe InGenius			
			Mean Ct	SD	%CV	% Agreement	Mean Ct	SD	%CV	%Agreement
Neg	HPV16-31	6	-	-	-	100%	-	-	-	100%
16+18 3xLoD		6	33.13	0.31	0.93	100%	32.68	0.48	1.47	100%
33+35 3xLoD		6	-	-	-	100%	-	-	-	100%
10xLoD		6	31.33	0.30	0.95	100%	30.86	0.33	1.07	100%
Neg	HPV18-45	6	-	-	-	100%	-	-	-	100%
16+18 3xLoD		6	35.22	0.49	1.38	100%	33.11	0.32	0.95	100%
33+35 3xLoD		6	-	-	-	100%	-	-	-	100%
10xLoD		6	33.69	0.57	1.68	100%	31.46	0.14	0.43	100%
Neg	HPV-HR1	6	-	-	-	100%	-	-	-	100%
16+18 3xLoD		6	-	-	-	100%	-	-	-	100%
33+35 3xLoD		6	34.72	0.43	1.23	100%	33.51	0.10	0.31	100%
10xLoD		6	31.52	0.58	1.83	100%	30.54	0.12	0.38	100%
Neg	HPV-HR2	6	-	-	-	100%	-	-	-	100%
16+18 3xLoD		6	-	-	-	100%	-	-	-	100%
33+35 3xLoD		6	33.38	0.39	1.18	100%	33.17	0.18	0.54	100%
10xLoD		6	31.48	0.39	1.23	100%	31.16	0.15	0.48	100%

An example of Inter-Session Repeatability (on two days) Ct results is shown in the table below.

Table 22 Inter-Session Repeatability test - Ct results (two days / one lot)

Sample	Target	N	ELITe BeGenius				ELITe InGenius			
			Mean Ct	SD	%CV	% Agreement	Mean Ct	SD	%CV	%Agreement
Neg	HPV16-31	12	-	-	-	100%	-	-	-	100%
16+18 3xLoD		12	33.11	0.44	1.33	100%	32.57	0.45	1.39	100%
33+35 3xLoD		12	-	-	-	100%	-	-	-	100%
10xLoD		12	31.21	0.37	1.19	100%	30.72	0.33	1.08	100%
Neg	HPV18-45	12	-	-	-	100%	-	-	-	100%
16+18 3xLoD		12	35.49	0.58	1.62	100%	33.12	0.37	1.11	100%
33+35 3xLoD		12	-	-	-	100%	-	-	-	100%
10xLoD		12	34.19	0.73	2.12	100%	31.82	0.50	1.56	100%
Neg	HPV-HR1	12	-	-	-	100%	-	-	-	100%
16+18 3xLoD		12	-	-	-	100%	-	-	-	100%
33+35 3xLoD		12	34.07	0.77	2.26	100%	32.65	0.91	2.80	100%
10xLoD		12	31.27	0.56	1.78	100%	30.23	0.35	1.15	100%
Neg	HPV-HR2	12	-	-	-	100%	-	-	-	100%
16+18 3xLoD		12	-	-	-	100%	-	-	-	100%
33+35 3xLoD		12	33.32	0.31	0.92	100%	32.84	0.37	1.13	100%
10xLoD		12	31.42	0.30	0.94	100%	31.07	0.18	0.59	100%

In the Repeatability test, the HPV PLUS ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 2.80% (and a maximum variability of target Tm values as %CV equal to 0.58%).

11.9 Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a pool of negative cervical specimen collected in ThinPrep spiked by reference materials (SiHa HPV16 cells, HeLa HPV18 cells, pHPV33 plasmid DNA and pHPV35 plasmid DNA).

The Ct results of Inter-Batch Reproducibility (on three lots) are shown in the table below.

Table 23 Inter-Batch Reproducibility test - Ct results (six days / three lots)

Sample	Target	N	ELITe BeGenius				ELITe InGenius			
			Mean Ct	SD	%CV	%Agreement	Mean Ct	SD	%CV	%Agreement
Neg	HPV16-31	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	32.88	0.55	1.68	100%	32.24	0.46	1.44	100%
33+35 3xLoD		36	-	-	-	100%	-	-	-	100%
10xLoD		36	30.81	0.52	1.68	100%	30.22	0.54	1.79	100%
Neg	HPV18-45	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	35.30	0.64	1.82	100%	33.39	0.41	1.24	100%
33+35 3xLoD		36	-	-	-	100%	-	-	-	100%
10xLoD		36	33.92	0.69	2.04	100%	31.94	0.48	1.51	100%
Neg	HPV-HR1	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	-	-	-	100%	-	-	-	100%
33+35 3xLoD		36	33.55	0.74	2.21	100%	32.49	0.67	2.08	100%
10xLoD		36	31.01	0.54	1.74	100%	30.12	0.33	1.10	100%
Neg	HPV-HR2	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	-	-	-	100%	-	-	-	100%
33+35 3xLoD		36	33.07	0.49	1.47	100%	32.71	0.33	1.02	100%
10xLoD		36	31.18	0.36	1.15	100%	30.97	0.21	0.66	100%

The results of Inter-Instrument Reproducibility (on three instruments) are shown in the table below.

Table 24 Inter-Instrument Reproducibility test - Ct results (six days / three lots / three instruments)

Sample	Target	N	ELITe BeGenius				ELITe InGenius			
			Mean Ct	SD	%CV	%Agreement	Mean Ct	SD	%CV	%Agreement
Neg	HPV16-31	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	32.47	0.46	1.42	100%	31.67	0.87	2.74	100%
33+35 3xLoD		36	-	-	-	100%	-	-	-	100%
10xLoD		36	30.35	0.45	1.50	100%	29.64	0.71	2.40	100%
Neg	HPV18-45	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	34.37	0.45	1.30	100%	33.21	0.42	1.28	100%
33+35 3xLoD		36	-	-	-	100%	-	-	-	100%
10xLoD		36	33.06	0.46	1.38	100%	31.80	0.37	1.18	100%
Neg	HPV-HR1	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	-	-	-	100%	-	-	-	100%
33+35 3xLoD		36	32.90	0.39	1.18	100%	32.73	0.41	1.26	100%
10xLoD		36	30.35	0.25	0.83	100%	30.09	0.30	1.01	100%
Neg	HPV-HR2	36	-	-	-	100%	-	-	-	100%
16+18 3xLoD		36	-	-	-	100%	-	-	-	100%
33+35 3xLoD		36	32.82	0.32	0.98	100%	32.72	0.25	0.76	100%
10xLoD		36	30.85	0.27	0.88	100%	30.79	0.24	0.77	100%

In the Reproducibility test, the HPV PLUS ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 2.74% (and a maximum variability of target Tm values as %CV equal to 0.41%).

11.10 Cross-contamination

The possible Cross-contamination during analysis was evaluated for the assay by testing 60 replicates of a pool of negative cervical specimens collected in ThinPrep alternated to 60 replicates of the same pool spiked by pHPV16 plasmid DNA (EG SpA) at a concentration of ~$1x10^7$ copies /mL in 5 sessions.

The results are reported in the following table.

Table 25 Cross-contamination test results on ELITe BeGenius

Samples	N	Positive	Negative	%Agreement
Positive	60	60	0	100%
Negative	60	0	60	100%

Cross-contamination was neither detected within sessions nor between sessions with the HPV PLUS ELITe MGB Kit.

11.11 Whole system failure

The whole system failure rate for the assay was evaluated by testing a panel of 100 negative cervical specimens collected in ThinPrep spiked at 3x LoD with HPV16 WHO International Standard (NIBSC).

The results are reported in the following table.

Table 26 Whole system failure test results on ELITe BeGenius

Samples	N	Positive	Negative	Whole system failure rate
Cervical samples spiked at 3x LoD	100	100	0	0%

None of the tested HPV16 positive samples gave false negative results using the HPV PLUS ELITe MGB Kit. The whole system failure rate was equal to 0%.

11.12 Clinical Performances

The Clinical Performances, taking into account Diagnostic Sensitivity and Diagnostic Specificity, were evaluated by analysing human cervical specimens placed in alcohol-based fixative for cytology in association with ELITe BeGenius.

As ELITe InGenius has equivalent analytical performances to ELITe BeGenius, the Clinical Performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Clinical Performances of the assay obtained in association with ELITe BeGenius are also applicable to ELITe InGenius.

The clinical specimens enrolled in the study were from women aged between 17 and 89 years with a previous cervical cytology or HPV molecular positive test results. The specimens were certified negative or positive with a validated reference method by an HPV-reference laboratory.

The Clinical Performances, as agreement with reference method, were evaluated by Cohen's kappa value and the results, for each HR-HPV channel, are summarized in the following tables.

Table 27 HPV16–31 clinical performances

		Reference method			AUC	Cohen's kappa
		Positive	Negative	Total		
HPV PLUS ELITe MGB Kit	Positive	56	0	56	98.4%	0.967
	Negative	2	65	67		
	Total	58	65	123		

Table 28 HPV HR2 clinical performances

		Reference method			AUC	Cohen's kappa
		Positive	Negative	Total		
HPV PLUS ELITe MGB Kit	Positive	54	3	57	94.2%	0.885
	Negative	4	59	63		
	Total	58	62	120		

Table 29 HPV HR1 clinical performances

		Reference method			AUC	Cohen's kappa
		Positive	Negative	Total		
HPV PLUS ELITe MGB Kit	Positive	49	0	49	96.6%	0.931
	Negative	4	65	69		
	Total	53	65	118		

Table 30 HPV18–45 clinical performances

		Reference method			AUC	Cohen's kappa
		Positive	Negative	Total		
HPV PLUS ELITe MGB Kit	Positive	53	1	54	97.5%	0.950
	Negative	2	64	66		
	Total	55	65	120		

In this analysis, the HPV PLUS ELITe MGB Kit generated an Area Under Curve (AUC) and a Cohen's Kappa value corresponding to an almost perfect agreement with results obtained with the reference method for all HR-HPV channel.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: cervical specimen placed in alcohol-based fixative for cytology (ThinPrep or Surepath).

Currently there are no data available concerning product performance with other clinical samples or other collection devices.

The results obtained with this product depend on proper 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 product.

Owing to its high analytical sensitivity, the Real Time PCR method used in this product is sensitive to contamination from positive clinical samples, positive controls and PCR products. Cross-contamination cause false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided 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 personal protective equipment 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 personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, PCR and detection of nucleic acids.

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 indicates that the target DNA is not detected in the DNA extracted from the sample; however, it cannot be excluded that the target DNA has a lower titer 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 for one target can be affected by the amplification of a second target (see Performance Characteristics).

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, insertions or deletions within the region of the DNA targeted by the product primers and probes may impair detection of target DNA.

Cross-reactivity is observed with HPV53 and HPV82, two HPV genotypes possibly carcinogenic (Group 2B) not included in the Intended Use of the product (see 11 PERFORMANCE CHARACTERISTICS page 17).

Partially inhibition is observed with HPV67 and HPV82, two HPV genotypes possibly carcinogenic (Group 2B) not included in the Intended Use of the product (see 11 PERFORMANCE CHARACTERISTICS page 17).

Inhibition is observed with Vaginal Moisturizer at high concentration (> 1% w/v, see 11 PERFORMANCE CHARACTERISTICS page 17).

This product is not validated as per Meijer’s HPV DNA screening guidelines.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid, or erroneous results 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. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

14 TROUBLESHOOTING

Table 31

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.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (about 3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (about 3 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 32

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.	Do not use the Negative Control for more than 1 session. 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, Racks, Inventory Block or Cooler Unit	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 33

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control, and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (about 3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Prepare a new aliquot of PCR Mix.

Table 33 (continued)

Invalid Sample reaction	
Possible Causes	**Solutions**
Inhibition due to interfering substances in the sample.	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 molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 34

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the positive control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 35

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session. - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 36

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination in preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix.

15 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Caution, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

16 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the “Summary of Safety and Performance” will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

17 NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Thermo Fisher Scientific. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

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

ELITe InGenius® and ELITe BeGenius® technologies are covered by patents and pending applications.

This limited license allows the person or entity to whom the product has been provided to use the product and data generated by the use of the product, solely for human diagnostics. Neither ELITechGroup S.p.A. nor its licensors grant any other licenses, expressed or implied for any other purposes.

Appendix A HPV PLUS ELITe MGB Kit used in association with Genius series® platforms

CAUTION

This document is a simplified version of the official instruction for use. Please refer to the complete document before use at www.elitechgroup.com.

INTENDED USE

The product **HPV PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the **genomic DNA of Human Papilloma Virus** 14 high-risk types as follow: detection and typing of the high-risk HPV 16, 18, 31, 45, detection of high-risk HPV HR1 group (33, 52, 58) and detection of high-risk HPV HR2 group, non-vaccinal types, (35, 39, 51, 56, 59, 66 and 68), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human cervical specimens placed in alcohol-based fixative for cytology.

The product is intended for use as an aid in the diagnosis of HPV infections, in patients with cervical cytology or HPV molecular positive test results. The results must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	E6/E7	FAM	HPV16-31
Target 2	E6/E7	AP690	HPV18-45
Target 3	E6/E7	AP639	HPV HR1
Target 4	E6/E7	AP593	HPV HR2
Internal Control	beta globin	AP525	IC

Validated matrix

Cervical specimen for liquid cytology, collected in ThinPrep® Pap Test PreservCyt® Solution (Hologic, Inc.)
Cervical specimen for liquid cytology, collected in BD SurePath™ Collection Vial 10 mL (Becton, Dickinson and C.)

Kit content and related products

HPV PLUS ELITe MGB Kit Kit (RTS402ING)		HPV PLUS - ELITe Positive Control (CTR402ING)	
 X 8		 X 3	
HPV PLUS PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube		HPV PLUS Positive Control 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles	
Maximum shelf-life:	**24 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

› ELITe InGenius instrument: INT030. › ELITe BeGenius instrument: INT040. › ELITe InGenius SP 200: INT032SP200. › ELITe InGenius SP200 Consumable Set: INT032CS. › ELITe InGenius PCR Cassette: INT035PCR. › ELITe InGenius Waste Box: F2102-000. › 300 µL Filter Tips Axigen: TF-350-L-R-S. › 1000 µL Filter Tips Tecan: 30180118.	**ThinPrep® Pap Test PreservCyt® Solution** (Hologic, Inc., code 70098-002, 20 mL of PreservCyt® Solution). **BD SurePath™ Collection Vial 10 mL** (Becton, Dickinson and Company, codes 491439 / 491438 / 491440, 10 mL of SurePath® Preservative Fluid).

ELITe InGenius and ELITe BeGenius Protocol

› Sample volume › Total elution volume	200 µL 100 µL	› Eluate PCR input volume › PCR Mix volume › Frequency of controls	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Target	Clinical performamnces	
		AUC	Cohen's kappa
Cervical specimens for liquid cytology	**HPV16-31**	**98.4%**	**0.967**
	HPV HR2	**94.2%**	**0.885**
	HPV HR1	**96.6%**	**0.931**
	HPV18–45	**97.5%**	**0.950**

Matrix	Target	Limit of Detection
Cervical samples collected in ThinPrep and Sure Path on ELITe BeGenius and ELITe InGenius	HPV16	1,700 (IU / mL)
	HPV18	1,700 (IU / mL)
	HPV31	1,700 (IU / mL)
	HPV33	2,000 (IU / mL)
	HPV45	1,700 (IU / mL)
	HPV35	1,700 (copies / mL)

Sample preparation

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**- 20 ± 10 °C**	**- 70 ± 15 °C**
Cervical specimens for liquid cytology	ThinPrep® Pap Test PreservCyt® Solution (Hologic, Inc.)	≤ 6 months	≤ 6 months	NR	NR
	BD SurePath™ Collection Vial 10 mL (Becton, Dickinson and C.)	≤ 3 months	≤ 6 months	NR	NR

NR: not recommended

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe InGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR), or PCR Only.

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: HPV PLUS ELITe_Cyt_Sense_ 200_100.	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix in the Inventory Block
7. Load PCR Cassettes, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: HPV PLUS ELITe_Cyt_Sense_200_100 or HPV PLUS ELITe_PC or HPV PLUS ELITe_NC	**5.** Select the method "PCR Only" and the sample position "Elution Tube"	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	8. Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe BeGenius® software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR), or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL"
4. Select the "Assay Protocol" of interest: HPV PLUS ELITe_Be_Cyt_Sense_200_100 Note: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix in the Reagent/ Elution Rack and insert it in the Cooler Unit
7. Load "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit.	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay Protocol” of interest: HPV PLUS ELITe_Be_Cyt_ Sense_200_100 or HPV PLUS ELITe_ Be_PC or HPV PLUS ELITe_Be_NC.	**5.** Load the PCR-Mix in the Reagent/ Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Rack” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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