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EMPOWERING IVD

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NOTICE of CHANGE dated 14/11/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«High Risk HPV ELITe Panel»
Ref. RTK402ING

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

- *Updating of matrix description*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

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High Risk HPV ELITe Panel

reagents for DNA Real Time amplification

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INTENDED USE

The **«High Risk HPV ELITe Panel»** product is a qualitative multiplex nucleic acids real time amplification assay for the detection and differentiation of the DNA of **Human Papilloma Virus 14 high-risk types in DNA samples extracted from cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium.**

The test specifically identifies types HPV16 and HPV18 while concurrently detects the rest of the high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The product is intended for in vitro diagnostic use as an aid in the diagnosis of HPV infections, together with patient's clinical data and other laboratory test results.

ASSAY PRINCIPLES

The assay consists of a multiplex Real Time amplification reaction by a programmable thermostat provided with a fluorescence detection optical system (real time amplification thermal cycler).

In each well, different amplification reactions are performed starting from DNA extracted from each sample under test, in order to amplify the HPV-HR targets:

- HPV16, revealed by specific probe detected by real time instrument in the channel for FAM,
- HPV18, revealed by specific probe detected by real time instrument in the channel for JOE/HEX
- HR-HPV (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), revealed by specific probe detected by real time instrument in the channel for Texas red/Cal fluor red 610.

The kit also amplifies an endogenous Internal Control based on ABL gene, detected by real time instrument in the channel for Cy5/Quasar 670.

Specific target DNA is amplified by forward, reverse primers and Taq polymerase. In real-time PCR, the amplified product is detected via fluorescent dye. The method relies on a DNA-based probe with a fluorescent reporter at one end, and a quencher of fluorescence at the opposite end of the probe. The close proximity of the reporter to the quencher prevents detection of its fluorescence; breakdown of the probe by the 5' to 3' exonuclease activity of the Taq polymerase breaks the reporter-quencher proximity and thus allows unquenched emission of fluorescence, which can be detected.

Fluorescence emission increases as the specific products of the amplification reaction increase and it is measured by the instrument during PCR run in real-time

The assay has been validated with the systems described in this user manual.

KIT DESCRIPTION

The **«High Risk HPV ELITe Panel»** kit provides the following components:

- **HR-HPV Reaction Mix**

A mixture of enzyme for specific region of Human papilloma virus and buffer, aliquoted into three test tubes (YELLOW cap) The mixture provides UNG (Uracil N-glycosidase) to prevent carry over contamination.

Each tube contains 550 µL of solution, sufficient for **32 tests** in association with **ELITe InGenius** when performing 4 sessions and **33 tests** in association with other systems.

- **HR-HPV Probe Mix**

A mixture with specific primer/probe for HPV and internal control, aliquoted into three test tubes (RED cap).

Each tube contains 320 µL of solution, sufficient for 32 tests in association with ELITe InGenius when performing 4 sessions and **33 tests** in association with other systems.

- **HR-HPV Positive Control**

A solution of plasmids for HPV in a stabilizing solution, aliquoted into two test tubes (BLUE cap).

Each tube contains 100 µL of solution, sufficient for **4 sessions** ("Extract + PCR" run mode) in association with the system «**ELITe InGenius**» and **10 sessions** in association with the other systems validated.

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

The product is sufficient for **100 tests in association with other systems**, including controls.

MATERIALS PROVIDED IN THE KIT

Component	Description	Quantity	Classification of hazards
HR-HPV Reaction Mix	Mixture of enzyme **YELLOW cap**	**3 x 550 µL**	-
HR-HPV Probe Mix	Mixture of primer and probe **RED cap**	**3 x 320 µL**	-
HR-HPV Positive Control	Plasmids for targets **BLUE cap**	**2 x 100 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- 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 (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).
- Programmable thermal cycler with optical fluorescence detection system 7500, 7500 Fast Real-Time PCR System (Applied Biosystems Inc) or CFX96™ Real-Time PCR Detection System–IVD (Bio-Rad Laboratories, Inc.) calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

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

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

- parameters for the amplification positive control **«HR-HPV ELITe_PC»**,
- parameters for the amplification negative control **«HR-HPV ELITe_NC»**,
- parameters for swab samples to be analyzed **«HR-HPV ELITe_CS_200_100»**.

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

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

For manual DNA extraction from samples to be analyzed, it is recommended the use of the commercialized product QIAamp DNA Mini Kit (Qiagen, Germany, Cat. # 51304) kit for the extraction of DNA from cellular and noncellular samples.

When a 7500, 7500 Fast Real-Time PCR System is used, it is required the use of generic product: «**MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL**» (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

When a CFX96™ Dx System is used, it is required the use of the generic products **«Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, white/clear»** (Bio-Rad, code HSP9601) microplates in association with «**MICROSEAL B ADHES SEAL,100/PK»** (Bio-Rad, code MSB1001) adhesive sealing sheets for real time amplification. Alternatively, an equivalent product can be used.

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.

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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, such as nucleic acids extraction, amplification and detection, 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.

When amplification session is manually setup, it is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product into the area designated for extraction / preparation of amplification reactions.

When amplification session is manually setup, it is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

When the extraction / amplification session is set up with the integrated instrument, it is necessary to have dedicated gowns, gloves and instruments.

The samples must be exclusively used for this type of analysis. Samples must be handled under a laminar airflow hood. Tubes containing different samples must never be opened at the same time. 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, free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. 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, free from DNA and RNA.

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

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

- **HR-HPV Reaction Mix**

The **HR-HPV Reaction Mix** must be stored at -20°C.
The **HR-HPV Reaction Mix** can be frozen and thawed for no more than **10 times**: further freezing/thawing cycles may cause a loss of product performances.

- **HR-HPV Probe Mix**

The **HR-HPV Probe Mix** must be stored at -20°C in the dark.
The **HR-HPV Probe Mix** can be frozen and thawed for no more than **10 times**: further freezing/thawing cycles may cause a loss of product performances.

- **HR-HPV Positive Control**

The **HR-HPV Positive Control** must be stored at -20°C.
The **HR-HPV Positive Control** can be frozen and thawed for no more than **10 times**: further freezing/thawing cycles may cause a loss of product performances.

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Cervical specimens collected in UTM medium

The cervical specimens for DNA extraction must be collected in UTM medium and cell from cervical specimens collected in UTM medium and identified according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) or refrigerated at at +2 / +8 °C for a maximum of two days.

The cervical specimens can be frozen and stored at -20 °C for a maximum of two months or at -70 °C until two years. Avoid freeze / thaw cycles. The freezing can lead to the inhibitor precipitation, cell lysis and pathogen nucleic acid degradation.

For the analysis with this product 0.2 mL of resuspended sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set»**.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in "Interfering substances" paragraph of "Performance characteristics" chapter.

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 amplification Positive Control, use the **HR-HPV Positive Control** reagent (provided with this kit) in association with protocols **HR-HPV ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with protocols **HR-HPV 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 in use.
Furthermore, the amplification controls must be re-run when:

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

Quality controls

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

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PROCEDURE

The procedure to use the **High Risk HPV ELITe Panel** with the **ELITe InGenius** system consists of three steps:

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

Verification of the system readiness

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

- switch on the **ELITe InGenius** instrument and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, HR-HPV Positive Control, HR-HPV Negative Control) were run in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are not amplification control results approved or valid, generate them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe kits, the **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **High Risk HPV ELITe Panel** is described in the table below:

Assay protocol for High Risk HPV ELITe Panel			
Name	**Matrix**	**Report**	**Characteristics**
HR-HPV ELITe_CS_200_100	Cervical specimen	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 5 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 **High Risk HPV ELITe 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 load the work session information. Refer to the instrument user's manual for more details.

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

A. Integrated run

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

1. If needed, thaw at room temperature (+18/25°C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph "samples and Controls"
2. Thaw at room temperature (+18 / 25 °C) the HR-HPV Reaction Mix (YELLOW cap) test tubes needed for the session, remembering that the content of each test tube is enough for 32 reactions. Mix gently, spin down the content for 5 seconds and test it immediately.
3. Thaw at room temperature (+18 / 25 °C) the HR-HPV Probe Mix (RED cap) tubes necessary for the session remembering that the content of each tube is sufficient to set up 32 reactions. Mix gently, spin down the content for 5 seconds and test it immediately.

Note: Thaw HR-HPV Probe Mix in the dark because the reagent is sensitive to the light.

4. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the complete reaction mixture HR-HPV PCR Mix and mark it in a recognizable manner with a permanent marker,
5. Calculate the volumes of the two components provided by kit that are needed for preparing the complete reaction mixture HR-HPV PCR Mix on the basis of the number of samples to be analysed, as described in the following table.

Note: In order to calculate the volumes of the two components it is necessary to define the number of samples (N) to be tested in the session and follow the table below.

Sample Number (N)	HR-HPV Reaction Mix	HR-HPV Probe Mix
1 ≤ N ≤ 4	(N+1) x 12.5 µL	(N+1) x 7.5 µL
5 ≤ N ≤ 8	(N+2) x 12.5 µL	(N+2) x 7.5 µL
9 ≤ N ≤ 12	(N+3) x 12.5 µL	(N+3) x 7.5 µL

6. Prepare the complete reaction mixture HR-HPV PCR Mix by adding to the dedicate 2 mL tube the calculated volumes of the two components.

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

Note: the complete reaction mixture **cannot** be stored, it is stable for two consecutive runs (in the same day of reconstitution of the reaction mixture), if loaded into the instrument (Inventory Area), but it's important to mix 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).

7. Mix gently, centrifuge the tube for 5 seconds to bring the content to the bottom and keep on ice;

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

8. Select "Perform Run" from the "Home" screen.
9. Ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
10. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
11. Select the Assay protocol to be used in the "Assay" column (i.e. HR-HPV ELITe_CS_200_100).
12. Ensure that the "Protocol" displayed is: "Extract + PCR".
13. Select the sample loading position in the "Sample Position" column and select "Sonication Tube". Click "Next" to continue the setup.
14. Load HR-HPV PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
15. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
16. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click "Next" to continue the setup.
17. Close the instrument door.
18. 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 in the "Elution tube" must be removed from the instrument, capped, identified and stored at -20 °C. Avoid spilling the extracted sample.

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

B. Amplification run

1. Thaw at room temperature (+18 / 25 °C) the test tubes containing the extracted samples. Mix gently, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep on ice,
2. Prepare the complete reaction mixture HR-HPV PCR Mix in sufficient volume for the session, as described in paragraph A. Integrated run (from point 2 to 7).

To set up an amplification run starting from extracted Nucleic Acids, carry out the following steps as per 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. HR-HPV ELITe_CS_200_100).
7. Select "PCR Only" in the "Protocol" column.
8. Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Click "Next" to continue the setup.
9. Load HR-HPV 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 Acids 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 in the "Elution tube" must be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the extracted sample.

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

C. Amplification run for Positive Control and Negative Control

1. Prepare the complete reaction mixture HR-HPV PCR Mix in sufficient volume for the session, as described in paragraph A. Integrated run (from point 2 to 7).
2. Thaw at room temperature (+18 / 25 °C) the test tubes containing the HR-HPV Positive Control. Mix by vortexing for 10 seconds, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep on ice,
3. Transfer at least 50 µL the molecular biology grade water 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. 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.
6. In the Track of interest, select the Assay protocol to be used in the "Assay" column.
7. For the Positive Control, select HR-HPV ELITe_PC, in the "Assay" column and fill in the lot number and expiry date of HR-HPV Positive Control.
8. For the Negative Control, select HR-HPV ELITe_NC, in the "Assay" column and fill in the lot number and expiry date of the molecular biology grade water.
9. Click "Next" to continue the setup.
10. Load HR-HPV PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
12. Load the "PCR Cassettes", the HR-HPV Positive Control tube and the tube with water for molecular biology (HR-HPV Negative Control) following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. 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 Positive Control must be removed from the instrument, capped 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 consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling 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 "Laboratory Information System" (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user's manual for more details.

The **ELITe InGenius** system generates the results with the product **High Risk HPV ELITe Panel** through the following procedure:

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

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A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of target genes ("g16", "g18", and "HR") in the Positive Control and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols "HR-HPV ELITe_PC" and "HR-HPV 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 Positive Control and Negative Control amplification results, specific for the amplification reagent lot, will expire **after 15 days**.

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

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

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

B. Validation of Sample results

The fluorescence signals emitted by the probes of target genes ("g16", "g18", and "HR") and by the probe of the endogenous Internal Control probe ("IC") in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol HR-HPV ELITe_CS_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**
HR-HPV Positive Control	APPROVED
2) Negative Control	**Status**
HR-HPV Negative Control	APPROVED

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

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

Result of sample run	Interpretation
g16: DNA Detected	The **DNA of HPV16 was detected** in the sample.
g18: DNA Detected	The **DNA of HPV18 was detected** in the sample.
HR: DNA Detected	The **DNA of HR-HPV was detected** in the sample.
g16: DNA Not Detected or below the LoD	The **DNA of HPV16 was not detected** in the sample. The sample is negative valid for this pathogen or its concentration is below the Limit of Detection of the assay.
g18: DNA Not Detected or below the LoD	The **DNA of HPV18 was not detected** in the sample. The sample is negative valid for this pathogen or its concentration is below the Limit of Detection of the assay.
HR: DNA Not Detected or below the LoD	The **DNA of HR-HPV was not detected** in the sample. The sample is negative valid for this pathogen or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Invalid assay result** caused by Internal Control failure due to incorrect extraction, inhibitors carry-over (or sampling error when endogenous Internal Control is used). The test should be repeated.

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

Note: when the endogenous Internal Control is used with cervical specimens, take into account that the number of cells in the sample could be not sufficient due to an incorrect sampling.

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

Samples reported as "g16 DNA Not Detected or below the LoD", "g18 DNA Not Detected or below the LoD", "HR-HPV DNA Not Detected or below the LoD" are suitable for analysis but it was not possible to detect targets DNA. In this case it cannot be excluded that targets DNA was 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 account all the clinical data and the other laboratory test outcomes concerning the patient.

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

C. Sample result reporting

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

The “Sample Report” shows the details of a work session sorted by selected sample (SID).
The “Track Report” shows the details of a work session sorted by selected Track.
The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

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PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The analytical sensitivity of this assay, as limit of detection, allows detecting the presence of about 10 copies in 5 µL of DNA added to the amplification reaction.
The analytical sensitivity of this assay, as detection limit, was tested using plasmidic DNA containing the amplification product at various concentrations.

Repeatability

The Repeatability, as intra-run imprecision, of this assay in association with the ELITe InGenius system was tested by performing 3 replicates of the Positive Control, tested through PCR process in the same session (1run/instrument, with a sample in three replicates/run). The test was performed on four different instruments.

The Ct values of each target were used to calculate the %CV in order to evaluate the Repeatability as imprecision intra run and inter-run.

A summary of results is shown in the tables below.

Intra - run Repeatability

Sample	session	Target g16			Target g18			Target HR		
		Ct Mean	SD	%CV	Ct Mean	SD	%CV	Ct Mean	SD	%CV
PC	Instrument 1	19.83	0.11	0.53	20.61	0.21	1.01	21.66	0.08	0.37
PC	Instrument 2	19.15	0.03	0.16	20.14	0.05	0.26	20.96	0.05	0.23
PC	Instrument 3	19.25	0.08	0.40	20.58	0.02	0.10	21.20	0.05	0.24
PC	Instrument 4	20.02	0.13	0.63	21.72	0.23	1.04	22.26	0.18	0.81

The Repeatability of this assay, as intra-run %CV, did not exceed 2% for all the targets.

The Repeatability, as inter-run imprecision, of this assay in association with the ELITe InGenius system was tested by performing 3 replicates of the Positive Control, tested through PCR process with the same operator, reagent lot, instrument, in the same environment and on two different days (1run/day per 2 days, with a sample in three replicates/run). The test was performed on two different laboratories.

The Ct values of each target were used to calculate the %CV in order to evaluate the Repeatability as imprecision intra run and inter-run.

A summary of results is shown in the tables below.

Inter - run Repeatability

Sample	session	Target g16			Target g18			Target HR		
		Ct Mean	SD	%CV	Ct Mean	SD	%CV	Ct Mean	SD	%CV
PC	run 1 + run 2	19.49	0.38	1.94	20.38	0.29	1.43	21.31	0.39	1.81
PC	run 3 + run 4	19.91	0.22	1.10	21.56	0.25	1.15	22.24	0.20	0.88

The Repeatability of this assay, as inter-run %CV, did not exceed 2% for all the targets.

Reproducibility

The Reproducibility, as "Instrument to Instrument" variability, of this assay in association with the ELITe InGenius system was tested by performing 6 replicates of the Positive Control, tested through PCR process with the same reagent lot, different operator, in two different laboratories, on different days and with two different instruments. (2 run/ instrument, per 2 instruments, with a sample in 3 replicates/run).

The Ct values of each target were used to calculate the %CV in order to evaluate the Reproducibility as imprecision inter-instruments.

A summary of results is shown in the tables below.

Inter - Instruments Reproducibility									
Sample	Target g16			Target g18			Target HR		
	Ct Mean	SD	%CV	Ct Mean	SD	%CV	Ct Mean	SD	%CV
PC	19.85	0.17	0.84	20.99	0.55	2.62	21.82	0.33	1.49

Reproducibility of this assay, as "Instrument to Instrument" %CV, did not exceed 3% for all the targets.

Diagnostic specificity: confirmation of negative samples

The Diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated by analyzing:

- 143 cervical samples that were negative for HPV16,
- 151 cervical samples that were negative for HPV18,
- 92 cervical samples that were negative for HR-HPV

The samples, previously tested with a MFDS certified real time amplification (OmniPlex-HPV, Genematrix, Korea), were tested by the assay in association with ELITe InGenius system in "Extract + PCR" mode.

The results, after invalid and discrepant resolution, are summarized in the following table.

Samples	N	positive	negative	invalid
cervical samples HPV16 negative	143	0	143	0
cervical samples HPV18 negative	151	0	151	0
cervical samples HR-HPV negative	92	1	91	0

In this test one cervical sample gave discrepant positive result. This result can be explained by the low titre of the pathogen that could be under the LoD of the reference method.

In this test, the assay diagnostic specificity was equal to 100% for HPV16, equal to 100% for HPV18 and equal to 98.9 % for HR-HPV.

The Internal Control Ct (IC Ct) cut-off value is set at 35 for each validated matrix.

Diagnostic sensitivity: confirmation of positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing:

- 29 cervical samples that were positive for HPV16,
- 21 cervical samples that were positive for HPV18,
- 76 cervical samples that were positive for HR-HPV

The samples, previously tested with a MFDS certified real time amplification product (OmniPlex-HPV, Genematrix, Korea), were tested by the assay in association with ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	positive	negative	invalid
cervical samples HPV16 positive	29	29	0	0
cervical samples HPV18 positive	21	21	0	0
cervical samples HR-HPV positive	76	76	0	0

In these tests, the assay diagnostic sensitivity was equal to 100 % for all the targets.

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7500, 7500 Fast Real-Time PCR System
CFX96™ Real-Time PCR Detection System–IVD

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Cervical specimens collected in UTM medium

The cervical specimens for DNA extraction must be collected in UTM medium medium and cell from cervical specimens collected in UTM medium and identified according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of two days or at +2 / +8 °C for a maximum of two days.

The cervical specimens can be frozen and stored at -20 °C for a maximum of two months or at -70 °C until two years. Avoid freeze / thaw cycles. The freezing can lead to the inhibitor precipitation, cell lysis and pathogen nucleic acid degradation.

Note: when you carry out the DNA extraction from cervical specimens by **QIAamp DNA Mini Kit**, please, follow the instructions for use manual.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in "Interfering substances" paragraph of "Performance characteristics" chapter.

Amplification controls

It is absolutely mandatory to validate each amplification session with a negative control reaction and a positive control reaction.

- as amplification Positive Control, use the **HR-HPV Positive Control** reagent (provided with this kit)
- as amplification Negative Control, use molecular biology grade water (not provided with this kit)

Quality controls

It is recommended to validate the whole analysis procedure of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material.

PROCEDURE

Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

When a **7500, 7500 Fast Real-Time PCR System** is used.

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

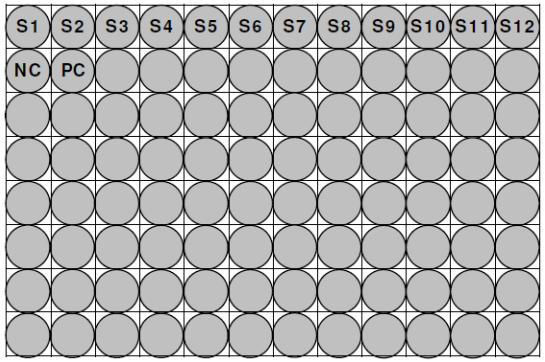
- switch on the real time thermal cycler, switch on the computer, run the dedicated software;
- set (Detector Manager) the "detector" for the *g16* probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "g16"
- set (Detector Manager) the "detector" for the *g18* probe with the "reporter" = "JOE" and the "quencher" = "none" (non fluorescent) and call it "g18"
- set (Detector Manager) the "detector" for the *HR* probe with the "reporter" = "Texas Red" and the "quencher" = "none" (non fluorescent) and call it "HR"
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "Cy5" and the "quencher" = "none" (non fluorescent) and call it "IC";

When a **CFX96™ Real-Time PCR Detection System–IVD** is used.

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software
- set (Detector Manager) the "detector" for the *g16* probe with the "reporter" = "FAM" and call it "g16"
- set (Detector Manager) the "detector" for the *g18* probe with the "reporter" = "HEX" and call it "g18"
- set (Detector Manager) the "detector" for the *HR* probe with the "reporter" = "Cal fluor red 610" and call it "HR"
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = " Quasar 670" and call it "IC";

Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

The set up of the qualitative analysis of 12 samples is shown, by way of example, here below



Legend: S1 - **S12**: Samples to be analysed; **NC**: Negative Control of amplification; **PC**: amplification positive control.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycler**:
- add to amplification stage the step (Add Step) of **Elongation at 72 °C**;

Thermal cycle				
Stage		**Temperatures**	**Timing**	**Cycle**
1	UDG-reaction	50 °C	2 min.	1 cycle
2	Pre-Denaturation	95 °C	10 min.	1 cycle
3	Denaturation	95 °C	15 sec.	45 cycles
	Annealing	54 °C	60 sec.	
	Elongation*	72 °C	30 sec.	

*Instrument setting; for 7500, 7500 Fast-select ON for data collection
for CFX-select 'Add plate Read to Step'

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Amplification set-up
(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw at room temperature the **HR-HPV Reaction Mix** tubes required for the session, remembering that each tube is sufficient for preparing **32 reactions**. Mix gently, spin down the content for 5 seconds and then test it immediately;
- take and thaw at room temperature the **HR-HPV Probe Mix** tubes required for the session, remembering that each tube is sufficient for preparing **32 reactions**. Mix gently, spin down the content for 5 seconds and then test it immediately;
- take and thaw at room temperature the **HR-HPV Positive Control** tube. Mix them gently, spin down the content for 5 seconds and then test it immediately;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powderless gloves and not to damage the wells.
- take the **Amplification Sealing Sheet** that will be used during the session, being careful to handle it with powderless gloves and not to damage,
- prepare one 2 mL molecular biology grade polypropylene tubes (not provided with this product) for the complete reaction mixture **HR-HPV PCR Mix** and mark them in a recognizable manner with a permanent marker,
- calculate the volumes of the two components provided by kit that are needed for preparing the complete reaction mixture **HR-HPV PCR Mix** on the basis of the number of samples to be analysed, as described in the following table.

Note: In order to calculate the volumes of the two components it is necessary to define the number of reactions (N) of the session by counting the number of the samples to be tested, a positive control and a negative control plus at least one reaction as safety margin.

Sample Number (N)	HR-HPV Reaction Mix	HR-HPV Probe Mix
N	(N+1) x 12.5 µL	(N+1) x 7.5 µL

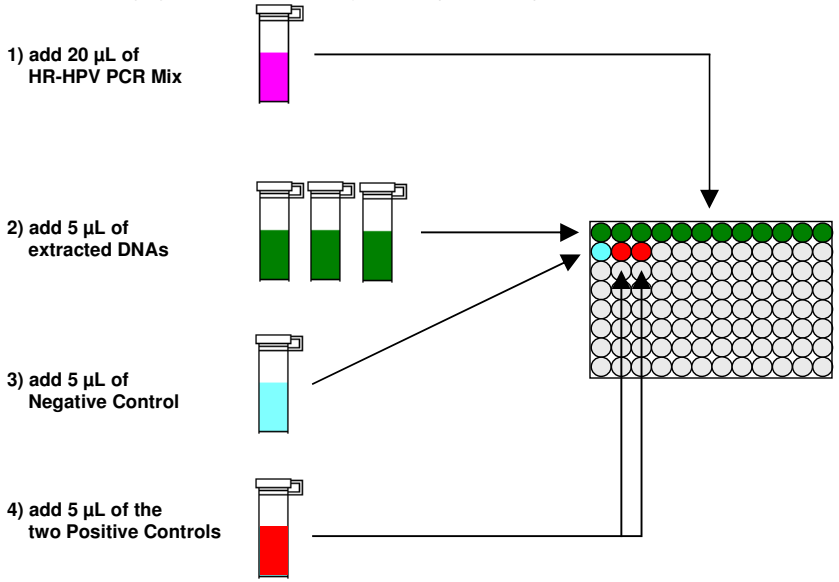
- prepare the complete reaction mixture **HR-HPV PCR Mix** by adding to the dedicated tube the calculated volumes of the two components.
- mix by pipetting without bubbles.

Set up the reactions as described below:

1. Accurately pipet **20 µL** of **HR-HPV PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.
2. Accurately pipet, by placing into the reaction mixture, **5 µL** of **DNA extract** from the first sample in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other samples of **extracted DNA**.
3. Accurately pipet, by placing into the reaction mixture, **5 µL** of **Molecular Biology grade water** (not provided by the product) in the well of **Amplification microplate** of the negative control of amplification, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **Molecular Biology grade water** three times into the reaction mixture. Avoid creating bubbles.
4. Accurately pipet, by placing into the reaction mixture, **5 µL** of **HR-HPV Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **HR-HPV Positive Control** three times into the reaction mixture. Avoid creating bubbles.
5. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.
6. Transfer the **Amplification microplate** into the real time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name.

Note: At the end of the thermal cycle the **Amplification microplate** with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. In order to avoid the spilling of the reaction products, the **Amplification Sealing Sheet must not be removed from the Amplification microplate**.

The following figure shows synthetically the set up of the amplification reaction.



Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific target probes ("g16.", "g18", "HR") and by the specific Internal Control probe ("IC") in the amplification reactions must be analysed by the instrument software.

Note: Data analysis is performed with the Instrument system software, and according to the manufacturer.

Before starting the analysis, set the threshold and baseline as follows. The values of fluorescence emitted by the specific probes in the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**, the cycle in which the fluorescence reached the **Threshold** value.

When a **7500, 7500 Fast Real-Time PCR System** is used:

Target	Threshold setting	Baseline	
		Start	End
HPV Type #16	20,000	3	15
HPV Type #18	20,000	3	15
HPV High Risk	20,000	10	15
IC	10,000	3	15

When a **CFX96™ Real-Time PCR Detection System–IVD** is used:

Target	Threshold setting	Baseline	
		Start	End
HPV Type #16	300	3	15
HPV Type #18	300	3	15
HPV High Risk	300	10	15
IC	100	3	15

Positive Control

In the amplification reactions of HR-HPV Positive Control the Ct values are used to validate the amplification and detection, as described in the following table:

HR-HPV Positive Control Reaction detector FAM "g16"	Assay result	Amplification / Detection
22 ± 3	POSITIVE	CORRECT

HR-HPV Positive Control Reaction detector JOE "g18"	Assay result	Amplification / Detection
23 ± 3	POSITIVE	CORRECT

HR-HPV Positive Control Reaction detector Texas Red "HR"	Assay result	Amplification / Detection
22 ± 3	POSITIVE	CORRECT

HR-HPV Internal Control Reaction detector Cy5 "HR"	Assay result	Amplification / Detection
*U.D or N/A	-	CORRECT

If the results of the **Positive Control** amplification reaction **Ct values exceed the standard ranges** or **Ct Undetermined** the target DNA has not been correctly detected. This means that problems occurred during the amplification or the detection step (incorrect dispensation of the reaction mix or of the positive controls, degradation of the reaction mix or of the positive controls, incorrect setting of the positive control position, incorrect setting of the thermal cycle), which may lead to incorrect results. The session is not valid and has to be repeated starting from the amplification step.

Negative Control

In the Negative control amplification reaction, the Ct value of HR-HPV is used to validate the amplification and the detection as described in the following table:

HR-HPV Negative Control Reaction detector FAM "g16"	Assay result	Amplification / Detection
*U.D or N/A	NEGATIVE	CORRECT

HR-HPV Negative Control Reaction detector JOE "g18"	Assay result	Amplification / Detection
*U.D or N/A	NEGATIVE	CORRECT

HR-HPV Negative Control Reaction detector Texas Red "HR"	Assay result	Amplification / Detection
*U.D or N/A	NEGATIVE	CORRECT

HR-HPV Internal Control Reaction detector Cy5 "HR"	Assay result	Amplification / Detection
*U.D or N/A	-	CORRECT

Use **Negative control** is recommended at the DNA isolation step to validate the procedure. All of signal should not be detected.

Note: if the result of amplification for negative control is different from "U.D or N/A" this means that problems occurred during the amplification. The session is not valid and needs to be repeated, starting from the amplification step.

Samples

The results as Ct of the amplification reactions of each **sample** are used as described in the following table:

#	Ct value				Positive or Negative				Results
	g16	g18	HR	IC	g16	g18	HR	IC	
1	≤45	≤45	≤45	≤35	+	+	+	+	g16, g18 and HR Positive
2	≤45	≤45	≤45	*U.D or N/A	+	+	+	-	**g16, g18 and HR Positive
3	≤45	≤45	U.D or N/A	≤35	+	+	-	+	g16 and g18 Positive
4	≤45	≤45	U.D or N/A	U.D or N/A	+	+	-	-	g16 and g18 Positive
5	≤45	U.D or N/A	≤45	≤35	+	-	+	+	g16 and HR Positive
6	≤45	U.D or N/A	≤45	U.D or N/A	+	-	+	-	g16 and HR Positive
7	≤45	U.D or N/A	U.D or N/A	≤35	+	-	-	+	g16 Positive
8	≤45	U.D or N/A	U.D or N/A	U.D or N/A	+	-	-	-	g16 Positive
9	U.D or N/A	≤45	≤45	≤35	-	+	+	+	g18 and HR Positive
10	U.D or N/A	≤45	≤45	U.D or N/A	-	+	+	-	g18 and HR Positive
11	U.D or N/A	≤45	U.D or N/A	≤35	-	+	-	+	g18 Positive
12	U.D or N/A	≤45	U.D or N/A	U.D or N/A	-	+	-	-	g18 Positive
13	U.D or N/A	U.D or N/A	≤45	≤35	-	-	+	+	HR Positive
14	U.D or N/A	U.D or N/A	≤45	U.D or N/A	-	-	+	-	HR Positive
15	U.D or N/A	U.D or N/A	U.D or N/A	≤39	-	-	-	+	Negative
16	U.D or N/A	U.D or N/A	U.D or N/A	U.D or N/A	-	-	-	-	***Invalid

Note:
*U.D: Undetermined
N/A: Not applicable
** When the target DNA is detected in a sample amplification reaction, the internal control (IC) may give the result as Ct Not applicable (N/A). In fact, the low-efficiency amplification reaction for the internal control may be displaced by competition from the high-efficiency amplification reaction for Target gene. In such a case, the sample is nevertheless suitable, and the positive result of the assay is valid.
*** This means that problems have occurred which may lead to incorrect results. It is not valid and the test needs to be repeated.

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PERFORMANCE CHARACTERISTICS

Analytical Sensitivity: limit of detection

The analytical sensitivity of this assay, as limit of detection, allows detecting the presence of about 10 copies in 5 µL of DNA added to the amplification reaction.
The analytical sensitivity of this assay, as detection limit, was tested using plasmidic DNA containing the amplification product at various concentrations.

Analytical Specificity: Cross Reactivity.

A total of 80 reference strains of Microorganism (Bacteria, Yeast, virus etc.), which have no concern with the detection targets of the kits, were used to evaluate the cross reactivity for this assay. All samples were negative to the test.

Interference

The potential for interference in this assay was assessed with substances that may be found in cervical specimens (erythrocyte, vaginal lubricant, douche, contraceptive jelly, anti-fungal cream, spermicide, mucus). Potential substances were spiked into a clinical sample. No interference in the performance of this assay was observed in presence of the substances.

Diagnostic sensitivity & specificity

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, and the diagnostic specificity of the assay, as confirmation of negative samples, was tested analysing a total of 265 clinical samples of known results (105 positive and 160 negative).

All positive HR-HPV type samples were confirmed to be positive, and all negative samples were confirmed to be negative.

Reproducibility

The reproducibility of this assay was tested for the following different conditions: lots, operators, operation dates, operation places.
14 types of reference DNA were tested based on the terms above. The tests results were determined within the acceptance criteria.

Repeatability

To evaluate the precision of this assay, the results obtained with several replicates of the same reference DNA sample were analysed. All the results obtained as 100% positive.

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PROCEDURE LIMITATIONS

Use this product only with clinical samples of cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium.

Do not use this product with samples containing mucin at high concentration: mucin inhibits the amplification reaction of nucleic acids and can cause invalid results.

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.

It is necessary to have separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products to prevent false positive results.

This product requires clothing and instruments to be used only for extraction / preparation of amplification reactions and only for amplification / detection of amplification products 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. 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 for 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 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, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

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TROUBLESHOOTING

Target DNA not detected in the Positive Control reactions	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells	Take care when dispensing reagents into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control dispensed.
Incorrect session setup on ELITe InGenius	Check the position of PCR Mix and positive control. Check the volumes of PCR Mix and positive control.
Probe degradation	Use a new aliquot of reaction mixture.
Standard degradation	Use a new aliquot of positive control.
Instrument setting error	Check the position settings for the positive control reactions on the instrument. Check the thermal cycle settings on the instrument.

Target DNA detected in the Negative control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative control and positive control into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius.	Check the position of PCR Mix and negative control. Check the volumes of PCR Mix and negative control.
Error while setting the instrument.	Check the position settings of the samples, negative control and positive control on the instrument.
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of the sterile bidistilled water.	Use a new aliquot of sterile water.
Contamination of the reaction mixture.	Use a new aliquot of reaction mixture.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Take care, by pipetting three times, when mixing samples, negative control and positive control into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase. Use the automatic baseline calculation by setting the "Auto Baseline" option.

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

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

High Risk HPV ELITe Panel
reagents for DNA Real Time amplification

REF **RTK402ING**

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *In vitro* Medical Device.

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.

EC REP Authorized representative in the European Community

NOTICE TO PURCHASER: LIMITED LICENSE

CONTACT INFORMATIONS

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WEB site: www.elitechgroup.com

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Fax: +82-31-460-9933
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High Risk HPV ELITe Panel used with ELITe InGenius®

Ref.: RTK402ING

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The **«High Risk HPV ELITe Panel»** product is a qualitative multiplex nucleic acids real time amplification assay for the detection and differentiation of the DNA of Human Papilloma Virus 14 high-risk types in DNA samples extracted from **cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium.**
The test specifically identifies types HPV16 and HPV18 while concurrently detects the rest of the high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The assay is CE-IVD validated in combination with the instrument **ELITe InGenius**.

B. Amplified sequence

Target	Gene	Fluorophore
HPV16	HPV16	FAM
HPV18	HPV18	JOE/HEX
HR-HPV	HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	Texas red/Cal fluor red 610
Internal Control	ABL gene	Cy5/Quasar 670

C. Validated matrix

- **Cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium**

D. Kit content

HR-HPV Reaction Mix	HR-HPV Probe Mix	HR-HPV Positive Control
		
3 tubes of 550 µL 96 reactions per kit 10 freeze-thaw cycles per tube	3 tubes of 320 µL 96 reactions per kit 10 freeze-thaw cycles per tube	2 tubes of 100 µL 96 reactions per kit 10 freeze-thaw cycles per tube

- **Maximum shelf-life: 18 months** **Storage: - 20°C**

E. Material required not provided in the kit

- **ELITe InGenius** instrument: INT030
- **ELITe InGenius SP200** Extraction Cartridge: INT032SP200
- **ELITe InGenius PCR Cassette** amplification cartridges: INT035PCR
- **ELITe InGenius SP200 Consumable Set** consumables for extraction: INT032CS
- **ELITe InGenius Waste Box:** F2102-000
- **300 µL Filter Tips:** TF-350-L-R-S
- **2 ml tube Sarstedt:** 72.694.005

F. ELITe InGenius protocol

Sample volume	200 µL	Unit of qualitative result	cp/reaction
Total eluate volume	100 µL	Frequency of controls	15 days
Sample PCR input volume	5 µL		
PCR Mix volume	20 µL		

G. Performance

Matrix	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Cervical Specimen	HPV16	10 copies/reaction	100% (29/29)*	100% (143/143)*
	HPV18	10 copies/reaction	100% (21/21)*	100% (151/151)*
	HR-HPV	10 copies/reaction	100% (76/76)*	98.9% (91/92)*

**confirmed samples/ tested samples*

H. Sample preparation

Sample Number (N)	HR-HPV Reaction Mix	HR-HPV Probe Mix
1 ≤ N ≤ 4	(N + 1) x 12.5 µL	(N + 1) x 7.5 µL
5 ≤ N ≤ 8	(N + 2) x 12.5 µL	(N + 2) x 7.5 µL
9 ≤ N ≤ 12	(N + 3) x 12.5 µL	(N + 3) x 7.5 µL

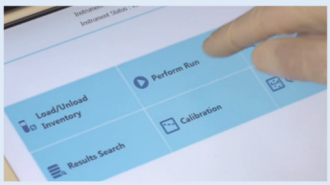
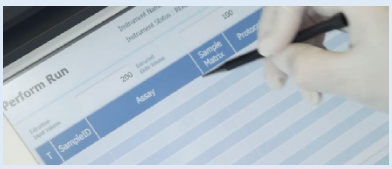
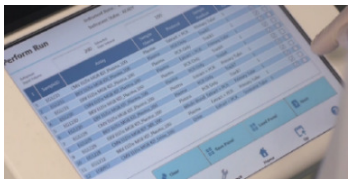
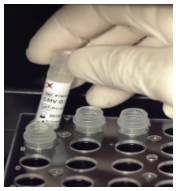
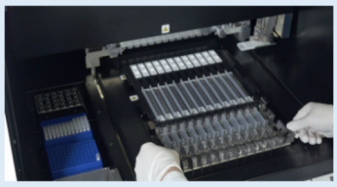
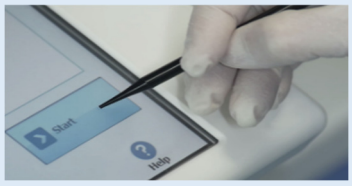
I. Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational mode are available: complete run, or extraction only, or PCR only.

Before analysis

1. Switch on ELITe InGenius Identification with username and password Select the mode “Closed”	**2.** Verify controls: HR-HPV ELITe Pos. and neg. controls in the “Control menu” NB: Both have been run, approved and not expired *NB:* Both have been run, approved and not expired	**3.** Thaw the HR-HPV PCR Mix components. Prepare the complete reaction mixture as described in the paragraph H Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select “Perform Run” on the touch screen 	**2.** Verify the extraction volumes: Input: “200 µL”, eluate: “100 µL” 	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID 
4. Select the “Assay protocol” of interest 	**5.** Select the sample position: Extraction tube 	**6.** Load the complete reaction mixture HR-HPV PCR Mix in the inventory block 
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, Extraction tube racks 	**8.** Close the door Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “PCR only” and set the sample position “Extra tube”	**6.** Load the extracted nucleic acid tubes in the rack n°4
7. Load the PCR cassette rack HR-HPV Reconstitution PCR-Mix in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position: Extraction tube	**6.** Load: Extraction cartridge, Elution tube, Tip cassette, Extraction tube racks
7. Close the door Start the run	**8.** Archive the eluate sample	

High Risk HPV ELITe Panel used with Open Platforms

Ref. RTK402ING

ELITechGroup
EMPOWERING IVD

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This document is available only in English.

A. Intended use

The **«High Risk HPV ELITe Panel»** product is a qualitative multiplex nucleic acids real time amplification assay for the detection and differentiation of the DNA of Human Papilloma Virus 14 high-risk types in DNA samples extracted from **cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium.**
The test specifically identifies types HPV16 and HPV18 while concurrently detects the rest of the high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The assay is CE-IVD validated in combination with the instruments **7500, 7500 Fast Real-Time PCR System and CFX96™ Real-Time PCR Detection System–IVD.**

B. Amplified sequence

Target	Gene	Fluorophore
HPV16	HPV16	FAM
HPV18	HPV18	JOE/HEX
HR-HPV	HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	Texas red/Cal fluor red 610
Internal Control	ABL gene	Cy5/Quasar 670

C. Validated matrix

› **Cervical specimens collected in UTM medium and cell from cervical specimens collected in UTM medium**

D. Kit content

HR-HPV Reaction Mix	HR-HPV Probe Mix	HR-HPV Positive Control
		
3 tubes of 550 µL 100 reactions per kit 10 freeze-thaw cycles per tube	3 tubes of 320 µL 100 reactions per kit 10 freeze-thaw cycles per tube	2 tubes of 100 µL 100 reactions per kit 10 freeze-thaw cycles per tube

Maximum shelf-life: 18 months **Storage: - 20°C**

E. Material required not provided in the kit

› 7500 RT PCR System ref: 4351105
› 7500 Fast RT PCR System ref: 4351106
› Biorad CFX96: Ref.1845097
› 2 mL Sarstedt tube (Ref. 72694005)
› Molecular biology grade water
› MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode
› 0.1 mL Hard-Shell 96-Well PCR Plates: Bio-Rad, code HSP9601
Microseal 'B' PCR Plate Sealing Film: Bio-Rad, code MSB1001

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
ABI 7500, 7500 Fast	Cervical specimens	10 copies/reaction	**100%** (105/105)*	**100%** (160/160)*
CFX96	Cervical specimens	10 copies/reaction	**100%** (105/105)*	**100%** (160/160)*

**confirmed samples/tested samples*

G. Sample preparation

Sample Number (N)	HR-HPV Reaction Mix	HR-HPV Probe Mix
N	(N + 1) x 12.5 µL	(N + 1) x 7.5 µL

H. Procedure

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Amplification - Settings of 7500, 7500 Fast Real-Time PCR System.

1. Switch on the thermal cycler
2. Set “g16” detector with “FAM” and quencher “none”
3. Set “g18” detector with “JOE” and quencher “none”
4. Set “HR” detector with “Texas Red” and quencher “none”
5. Set “IC” detector with “Cy5” and quencher “none”
6. Set up the thermal profile as indicated. Fluorescence acquisition must be set during Elongationstep at 72°C

Stage	Temperature	Timing
UDG-reaction	50°C	2 min
Pre-Denaturation	95°C	10 min
Denaturation	95°C	15 sec
Annealing	54°C	60 sec
Elongation	72°C	30 sec
45 cycles		

Amplification - Settings of CFX96™ Real-Time PCR Detection System–IVD.

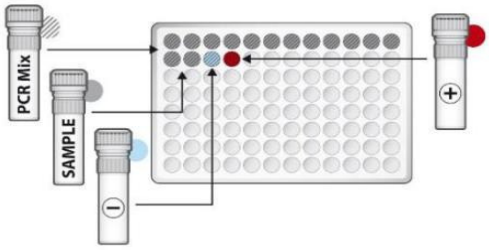
1. Switch on the thermal cycler
2. Set “g16” detector with “FAM”
3. Set “g18” detector with “HEX”
4. Set “HR” detector with “Cal fluor red 610”
5. Set “IC” detector with “Quasar 670”
6. Set up the thermal profil as indicated. Fluorescence acquisition must be set during Elongationstep at 72°C

Stage	Temperature	Timing
UDG-reaction	50°C	2 min
Pre-Denaturation	95°C	10 min
Denaturation	95°C	15 sec
Annealing	54°C	60 sec
Elongation	72°C	30 sec
45 cycles		

Amplification - PCR Set-up

1. Thaw the HR-HPV PCR Mix components.
2. Prepare the complete reaction mixture HR-HPV PCR Mix as described in the paragraph G
3. Mix gently and spin-down
4. Pipet **20 µL** of HR-HPV PCR Mix in all microplate wells in use
5. Add **5 µL** of extracted DNA in sample wells, **5 µL** of molecular grade water in Negative Control well, and **5 µL** of the HR-HPV Positive Controls

   Each one has to be mixed by pipetting 3 times into the reaction mixture
6. Seal the microplate with the amplification sealing sheet
7. Transfer the microplate in the thermal cyclerand start



Amplification - Threshold for qualitative analysis 7500, 7500 Fast Real-Time PCR System

Target	Threshold setting	Baseline	
		Start	End
HPV Type #16	20,000	3	15
HPV Type #18	20,000	3	15
HPV High Risk	20,000	10	15
IC	10,000	3	15

Amplification - Threshold for qualitative analysis CFX96™ Real-Time PCR Detection System–IVD

Target	Threshold setting	Baseline	
		Start	End
HPV Type #16	300	3	15
HPV Type #18	300	3	15
HPV High Risk	300	10	15
IC	100	3	15

Interpretation - Qualitative results

#	Ct value				Positive or Negative				Results
	g16	g18	HR	IC	g16	g18	HR	IC	
1	≤45	≤45	≤45	≤35	+	+	+	+	g16, g18 and HR Positive
2	≤45	≤45	≤45	*U.D or N/A	+	+	+	-	**g16, g18 and HR Positive
3	≤45	≤45	U.D or N/A	≤35	+	+	-	+	g16 and g18 Positive
4	≤45	≤45	U.D or N/A	U.D or N/A	+	+	-	-	g16 and g18 Positive
5	≤45	U.D or N/A	≤45	≤35	+	-	+	+	g16 and HR Positive
6	≤45	U.D or N/A	≤45	U.D or N/A	+	-	+	-	g16 and HR Positive
7	≤45	U.D or N/A	U.D or N/A	≤35	+	-	-	+	g16 Positive
8	≤45	U.D or N/A	U.D or N/A	U.D or N/A	+	-	-	-	g16 Positive
9	U.D or N/A	≤45	≤45	≤35	-	+	+	+	g18 and HR Positive
10	U.D or N/A	≤45	≤45	U.D or N/A	-	+	+	-	g18 and HR Positive
11	U.D or N/A	≤45	U.D or N/A	≤35	-	+	-	+	g18 Positive
12	U.D or N/A	≤45	U.D or N/A	U.D or N/A	-	+	-	-	g18 Positive
13	U.D or N/A	U.D or N/A	≤45	≤35	-	-	+	+	HR Positive
14	U.D or N/A	U.D or N/A	≤45	U.D or N/A	-	-	+	-	HR Positive
15	U.D or N/A	U.D or N/A	U.D or N/A	≤39	-	-	-	+	Negative
16	U.D or N/A	U.D or N/A	U.D or N/A	U.D or N/A	-	-	-	-	***Invalid

Note:

*U.D: Undetermined

N/A: Not applicable

** When the target DNA is detected in a sample amplification reaction, the internal control (IC) may give the result as Ct Not applicable (N/A). In fact, the low-efficiency amplification reaction for the internal control may be displaced by competition from the high-efficiency amplification reaction for Target gene. In such a case, the sample is nevertheless suitable, and the positive result of the assay is valid.

*** This means that problems have occurred which may lead to incorrect results. It is not valid and the test needs to be repeated.