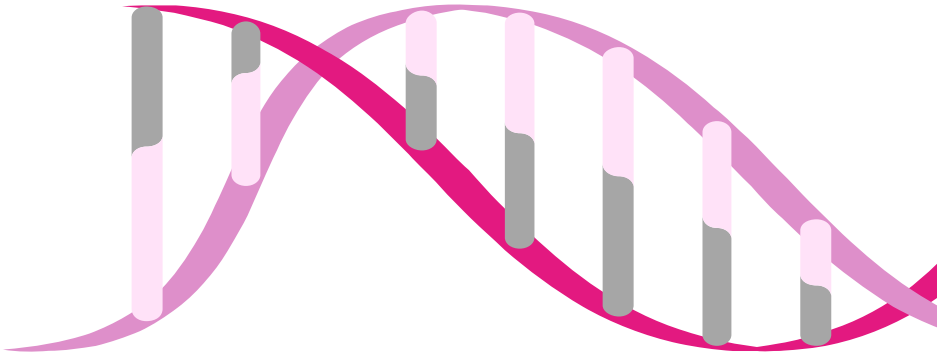
For professional use only



ULISSE BioMed

We build using DNA: the molecule of life

Sagitta™

HPV Selfy HR

INSTRUCTIONS FOR USE

Multiplex real-time PCR assay for the detection and genotyping of 14 high-risk HPV types from cervical swab, vaginal swab and liquid based cervical cytology specimens.

14 high-risk HPV genotypes:

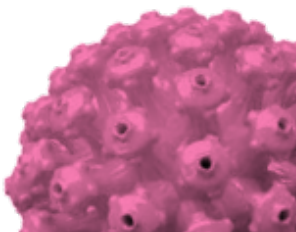
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

CE IVD

REF UBM0013

HIGH SENSITIVITY & SPECIFICITY

Multiplex real-time PCR with high sensitivity and specificity by **utilization of SAGITTA™ patented technology**



Version 8 | January 2024

DISCLAIMER

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1. Product description

Intended use

"HPV Selfy HR" is an *in vitro* diagnostic (IVD) medical device intended for the qualitative multiplex detection and differentiation of nucleic acids from 14 high-risk Human Papillomaviruses (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) with real-time polymerase chain reaction (PCR) from cervical swab, vaginal swab and liquid based cervical cytology specimens.

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

Positive results indicate the presence of DNA of one or more of the 14 HPV types, but do not provide information on the presence of bacterial infection or of co-infections with other viruses, included other oncogenic or not-oncogenic HPV types.

Negative results do not preclude HPV infection and therefore, HPV Selfy HR cannot be the only diagnostic tool to evaluate possible treatments and investigations. Negative results should be combined with clinical observations, patient history and epidemiological information.

Principles and procedure overview

The HPV Selfy HR assay is based on the proprietary SAGITTA technology of Ulisse Biomed S.p.A. which enables simultaneous detection of multiple pathogens in a single fluorescence channel on real-time PCR instruments by melting curve analysis.

HPV Selfy HR is a multiplex real-time PCR assay that permits the simultaneous amplification, detection and differentiation of target nucleic acids of 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) as well as Internal Control (IC).

HPV Selfy HR performs the amplification reaction starting from DNA extracted from each sample under test. HPV Selfy HR is also compatible with unpurified samples upon pre-treatment with Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014; not included in the present kit), a reagent that allows to avoid DNA extraction.

An Internal Control (IC) is incorporated into the product as an endogenous whole process control in order to monitor nucleic acid isolation, and to check for possible PCR inhibition. The IC is amplified simultaneously with the target nucleic acids. HPV Selfy HR uses Human ß-globin as an endogenous IC which can ensure purification of DNA, verification of PCR reaction and clarification of cell adequacy from each specimen.

The HPV Selfy HR assay consists of two PCR reactions:

- the first permitting the simultaneous amplification of target DNA of 14 high-risk HPV types (HR HPV PCR Mix);
- the second permitting the amplification of target DNA of the Human ß-globin (ß-globin PCR Mix).

In PCR, efficiency can be reduced by inhibitors that may be present in the clinical specimens.

Storage and handling

The components of HPV Selfy HR should be stored at a temperature between -25 °C and -15 °C, in an upright position and away from light. All components are stable under recommended storage conditions until the expiry date stated on the label. Repeated thawing and freezing should be avoided, as this may reduce the sensitivity. HPV Selfy HR can be frozen and thawed for no more than 6 times; further freezing/thawing cycles may cause a loss of product performance. If the reagents are to be used only intermittently, they should be frozen in aliquots in RNase/DNase free tubes.

Materials provided

The reagents contained in one kit of HPV Selfy HR (Ulisse Biomed, S.p.A.; code #UBM0013-050) are sufficient for 18 tests, including controls, in optimal reagent consumption conditions (at least 3 tests per session) when used with ELITe InGenius® system.

The reagents contained in one kit of HPV Selfy HR (Ulisse Biomed, S.p.A.; code #UBM0013-050) are sufficient for 50 tests in association with other systems, including controls.

HPV Selfy HR (REF UBM0013-050)			
Contents	Volume	Description	Color
HR HPV Mix	1 X 0.35 mL	Buffered solution containing synthetic DNA for the specific amplification of 14 high-risk HPV types.	Yellow
β-globin Mix	1 X 0.35 mL	Buffered solution containing synthetic DNA for the specific amplification of β-globin.	Pink
Reaction Mix (DNA)	1 X 1.50 mL	Buffered solution containing amplification and detection agents.	White
$MgCl_2$ 25mM solution	1 X 0.10 mL	Magnesium chloride 25mM solution.	Green
Positive Control HPV	1 X 0.20 mL	Buffered solution containing synthetic DNA segments of HPV strains and β-globin (5,000 copies/µl).	Red
Negative Control	1 X 0.20 mL	Molecular-biology grade water.	White

Materials required but not provided

A. Materials required for every compatible system:

The following materials are required to use HPV Selfy HR on every compatible system:

- molecular-biology grade water, RNase and DNase free.
- Nucleic acid isolation kit (see Nucleic acid isolation).
- 1.5 mL and 5 mL polypropylene capped tubes, sterile, RNase and DNase free.
- Precision calibrated pipettes capable of dispensing 2-20 µl (0.1-0.2 µl increment), 20-200 µl (0.1-0.2 µl increment), and 100-1,000 µl (1-2 µl increment).
- Anti-aerosol, single use, low-retention sterile filter tips for precision pipettes of 2-20 µl, 20-200 µl, and 100-1,250 µl, nuclease free.
- Desktop centrifuge.
- Vortex mixer.
- Class II laminar airflow biological hood.
- CFX96™ Real-time PCR detection system (Bio-Rad Laboratories, Inc.), QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.), AriaDx Real-time PCR System (Agilent Technologies, Inc.), ELITe InGenius® (ELITechGroup, S.p.A.) or HYRIS bCUBE™ (HYRIS, S.r.l.) calibrated following manufacturer's instructions.
- Ice.
- Disposable nitrile powder-free gloves, or similar material, and adequate personal protective equipment.

B. Materials required for CFX96™ Real-time PCR detection system (Bio-Rad Laboratories, Inc.)

For use with CFX96™ Real-time PCR detection system (Bio-Rad Laboratories, Inc.) instrument the following materials are required:

- Multiplate™ 96-Well PCR Plates, low profile, unskirted, clear (Bio-Rad Laboratories, Inc.; code #MLL9601).
- Microseal 'A' Film (Bio-Rad Laboratories, Inc.; code #MSA5001), Microseal 'B' Film (Bio-Rad Laboratories, Inc.; code #MSB5001) or Microseal 'C' Film (Bio-Rad Laboratories, Inc.; code #MSC5001).
- 1x Phosphate Buffered Saline solution (PBS).

C. Materials required for QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.)

For use with QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.) instrument the following materials are required:

- MicroAmp™ Optical 96-Well Reaction Plate (Applied Biosystems, Inc.; code #N8010560).
- MicroAmp™ Optical Adhesive Film (Applied Biosystems, Inc.; code #4311971).
- 1x Phosphate Buffered Saline solution (PBS).

D. Materials required for AriaDx Real-time PCR System (Agilent Technologies, Inc.)

For use with AriaDx Real-time PCR System (Agilent Technologies Inc.) instrument the following materials are required:

- 96-well plates, skirted and low profile (Agilent Technologies, Inc.; code #401490).
- Adhesive plate seals (Agilent Technologies, Inc.; code #401492).
- 1x Phosphate Buffered Saline solution (PBS).

E. Materials required for ELITe InGenius® (ELITechGroup, S.p.A.)

For use with ELITe InGenius® (ELITechGroup, S.p.A.) instrument the following materials are required:

- extraction cartridges "ELITe InGenius® SP 200" (ELITechGroup, S.p.A.; code #INT032SP200).
- Consumables for extraction "ELITe InGenius® SP 200 Consumable Set" (ELITechGroup, S.p.A.; code #INT032CS).
- Amplification cartridges "ELITe InGenius® PCR Cassette" (ELITechGroup, S.p.A.; code #INT035PCR).
- Tips "300 µL Filter tips Axygen" (Axygen BioScience, Inc.; code #TF-350-L-R-S),
- Boxes "ELITe InGenius® Waste Box" (ELITechGroup, S.p.A.; code #F2102-000).
- Sarstedt™ screw cap 2 mL Micro Tube (Sarstedt, AG & Co.; code #72.694.006).
- Sarstedt™ screw cap 0,5 mL Micro Tube (Sarstedt, AG & Co.; code #72.730.005).

Furthermore, the following specific Assay Protocols (ELITechGroup, S.p.A.) are required:

- Assay Protocols for the amplification positive control "Ulisse HPV Mix_PC_00" and "Assay_Ulisse Bglobin Mix_PC_00".
- Assay Protocols for the amplification negative control "Ulisse HPV Mix_NC_00" and "Assay_Ulisse Bglobin Mix_NC_00".
- Assay Protocols for the samples to be analyzed "Ulisse HPV Mix_CYT_200_200_00", "Ulisse HPV Mix_CS_200_200_00", "Ulisse Bglobin Mix_CYT_200_200_00" and "Ulisse Bglobin Mix_CS_200_200_00".

F. Materials required for HYRIS bCUBE™ (HYRIS, S.r.l.)

For use with HYRIS bCUBE™ (HYRIS, S.r.l.) instrument the following materials are required:

- 16 or 36 wells cartridges for HYRIS bCUBE™ with adhesive plate seals (HYRIS, S.r.l.; code #HyCT16.01 or #HyCT36.01).
- HYRIS bAPP™: a web user interface compatible with smartphones, tablets, laptops and PCs of any make and model.
- 1x Phosphate Buffered Saline solution (PBS).

Recommended materials

External Positive Controls are available from Microbix Biosystems Inc (https://microbix.com/home.php); such controls consist of inactivated high-risk human papillomavirus (HPV) whole-genomes and human cells in 1 mL of preservative solution. Prior to use, each 1 ml control should be combined with 3 mL of pre-qualified, HPV negative liquid-based cytology specimens. Available Positive Controls include:

- Microbix REDx™ HPV 16 Positive Control (Microbix catalog no. RED-62-16)
- Microbix REDx™ HPV 18 Positive Control (Microbix catalog no. RED-62-18)
- Microbix REDx™ HPV 31 Positive Control (Microbix catalog no. RED-62-31)
- Microbix REDx™ HPV 33 Positive Control (Microbix catalog no. RED-62-33)
- Microbix REDx™ HPV 45 Positive Control (Microbix catalog no. RED-62-45)
- Microbix REDx™ HPV 51 Positive Control (Microbix catalog no. RED-62-51)
- Microbix REDx™ HPV 66 Positive Control (Microbix catalog no. RED-62-66)
- Microbix REDx™ HPV Negative Control (Microbix catalog no. RED-99-M1)
- Microbix REDx™ HPV hr-HPV Negative Control (Microbix catalog no. RED-62-67)

2. 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.
- Wear suitable protective clothes and gloves; protect eyes and face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Carefully wash hands after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with the regulations in force.
- Carefully read all the instructions provided with the product before running the assay.
- While running the assay, follow the instructions provided with the product.
- Do not use the product after the indicated expiry date.
- Do not use the product if, upon receipt, the package is damaged, or the seal is broken.
- Only use the reagents provided with the product and those recommended by the manufacturer.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

- Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Lab coats, gloves and tools dedicated to work session setup are needed.
- The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose.
- The PCR cassettes or plates 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.
- While running the assay, follow the instruction contained in the Human Papillomavirus laboratory manual published by the World Health Organization.

3. Protocol

Specimen collection, storage and transport

A. Specimen Collection

Liquid based cervical cytology specimen

Cervical specimen collected in ThinPrep® media using an endocervical brush/spatula has been validated for use with HPV Selfy HR. Follow the manufacturer's instructions for collecting cervical specimen.

Cervical swab specimen

For the collection of cervical swab specimen, please use following materials according to manufacturer's instructions:

- FLOQSwab® cone-shaped tip 80 mm (Copan Italia, S.p.A.; code #52980C) for endo-esocervical specimens collection performed by a physician.

Vaginal swab specimen

For the self-collection of vaginal swab specimen, please use following materials according to manufacturer's instructions:

- FLOQSwab® regular plus, rounded tip, peelable barcode, no breaking point (Copan Italia, S.p.A.; code #5E046S) for self-collection of vaginal specimens.

B. Specimen Storage

The sensitivity of the assay may decrease if specimen is repeatedly frozen and thawed or stored for a long period of time. Nucleic acids should be extracted from the specimen as quickly as possible.

Liquid based cervical cytology specimen

Cervical cell specimen collected in ThinPrep® medium may be stored at 2 ~ 8 °C for up to 6 weeks.

Cervical and vaginal swab specimens

If the cervical and vaginal swab specimens are not processed directly after their receipt in the laboratory, they have to be stored at -15° ~ -25 °C and have to be processed within one month.

C. Specimen Transport

To ensure a high quality of sample, specimens should be transported as soon as possible at indicated temperature.

Liquid based cervical cytology specimen

Cervical cell specimen collected in ThinPrep® medium can be transported at 2 ~ 25 °C.

Cervical and vaginal swab specimens

Cervical and vaginal swab specimens shall be preferably transported cooled, but they can be transported at room temperature (~ + 25 °C) for a period no longer than 7 days. Cervical and vaginal swab specimens should be shipped to a laboratory as soon as possible after collection, following the laboratory instructions for transports. The samples should be transported following also the local and national instructions for the transport of pathogen material.

Procedure for CFX96™, QuantStudio™ 5, Agilent AriaDx

The procedure to use HPV Selfy HR with the abovementioned real-time PCR systems consists of six steps:

A. nucleic acid isolation.
B. Preparation of amplification PCR mixes.
C. PCR plate assembly.
D. Real-time PCR instrument setup.
E. Interpretation of tests results.
F. Quality control.

A. Nucleic acid isolation

Various manufacturers offer nucleic acid isolation kits. Use the right amount of sample according to the protocol in use. The following isolation kits have been validated for use with HPV Selfy HR.

a. Preparation of liquid based cervical cytology specimens

Before pretreatment with Ulisse Faster DNA or DNA extraction, liquid based cervical cytology specimens stored in Thin Prep® have to be prepared as indicated hereby:

- vortex the Thin Prep® vial for at least 30 seconds to homogenize the sample.
- Transfer 1.5 mL of liquid based cervical cytology specimen from the original Thin Prep® vial into a 1.5 mL Eppendorf tube.
- Centrifuge the tube at 9,000 g for 3 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet. Excessive leftover of Thin Prep® solution could cause inhibition of the following PCR reaction.
- Add 1 mL of 1x Phosphate Buffered Saline solution (PBS) to the cell pellet and place the tube on the vortex for at least 30 seconds.
- Centrifuge the tube at 9,000 g for 3 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet.
- Resuspend in 80 µL of molecular-biology grade water.

The prepared samples can be now pretreated with Ulisse Faster DNA or extracted with DNA extraction kits following manufacturer's instructions.

b. Preparation of cervical and vaginal swab specimens

Before pretreatment with Ulisse Faster DNA or DNA extraction, vaginal or cervical swab specimens have to be resuspended as indicated hereby:

- use a pipette with a disposable tip to transfer 2 mL of molecular-biology grade water into the 5 mL tube.
- Immerse the swab in the water with a series of rapid vertical movements; subsequently and without being immersed, the plug must be rotated by pressing it against the walls of the tube in order to facilitate the release of as much material as possible.
- Make the suspension homogeneous by vortexing it for 10-20 seconds so that no precipitate is visible.
- The prepared samples can be now pretreated with Ulisse Faster DNA or extracted with DNA extraction kits following manufacturer's instructions.

c. Compatible isolation kits

The following isolation kits have been validated for use with HPV Selfy HR:

- QIAamp® DNA Mini Kit (Qiagen, Inc.; code #51304); elute in molecular-biology grade water.
- Reliaprep™ Blood gDNA Miniprep System (Promega, Corp.; code #A5082).
- Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014).

B. Preparation of amplification PCR mix

Thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.

Prepare two 1.5 mL polypropylene capped tubes which will contain the HR HPV PCR Mix and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.

Prepare the HR HPV PCR Mix and the ß-globin PCR Mix: for each session, combine the following components sufficient for the number of samples to be tested plus one Positive Control and one Negative Control. All volumes include 10% overage for pipette error.

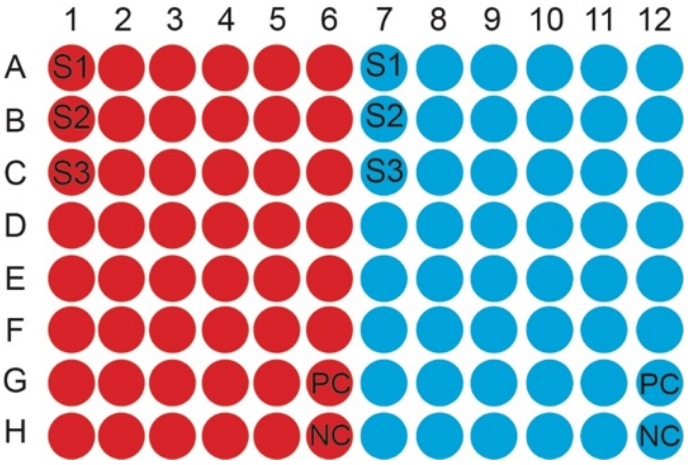
HR HPV PCR Mix		
Reagent	Volume per sample or control	Volume for "n" samples plus 2 controls
Reaction Mix (DNA)	12.00 µL	13.20 x (n + 2) µL
$MgCl_2$ 25mM solution	0.60 µL	0.66 x (n + 2) µL
HR HPV Mix	5.40 µL	5.94 x (n + 2) µL
Total Volume	18.00 µL	-
ß-globin PCR Mix		
Reagent	Volume per sample or control	Volume for "n" samples plus 2 controls
Reaction Mix (DNA)	12.00 µL	13.20 x (n + 2) µL
$MgCl_2$ 25mM solution	0.60 µL	0.66 x (n + 2) µL
ß-globin Mix	5.40 µL	5.94 x (n + 2) µL
Total Volume	18.00 µL	-

At the end, vortex the PCR mix and spin them briefly, avoiding the formation of bubbles.

C. PCR plate assembly

Load 18 µL of HR HPV PCR Mix, and 18 µL of ß-globin PCR Mix for each sample in two separate wells.

Load twice 2 µL of each biological sample (S1, S2, S3, etc), of Positive Control (PC) and of Negative Control (NC): once in the HR HPV PCR Mix-loaded well and once in the ß-globin PCR Mix-loaded well, as indicated in the figure below.



Seal the PCR plate using adequate adhesive seals following manufacturer's instructions.

D. Real-time PCR instrument setup

Template files for compatible real-time PCR systems are available upon request. To load the template file on the real-time PCR instrument, follow instrument software's instructions.

Before starting the run, insert the samples names.

If you do not want to use the template files or the template files are not available for the instrument, please setup the instrument and protocols according to the following indications:

Parameter	Setting
Volume	20 µL
Cover (Lid temperature)	105 °C
Reporter for each mix	SYBR
Quencher for each mix	None
Passive reference[1]	None

Step	Stage		Time	Temperature	Data collection
PCR	Polymerase activation		30 sec	98.0 °C	-
	Denaturation	repeat 36 cycles	5 sec	98.0 °C	-
	Annealing		10 sec	61.5 °C	-
	Extension		1 sec	72.0 °C	yes
Melting curve	Denaturation		15 sec	95.0 °C	-
	Start melting		60 sec	60.0 °C	-
	Optimal ramp increment / Soak time[2]		0.1°C / 3 sec		yes
	End melting		1 sec	95.0 °C	-

E. Interpretation of tests results

The recorded values of the fluorescence in the amplification reactions must be analyzed by the instrument software. Data analysis is performed with the instrument system software, and according to manufacturer's instruction. The values of fluorescence allow determining the threshold cycle (Ct), the cycle in which the fluorescence reached the threshold value. Before starting the analysis, set the threshold as follows:

PCR Instrument	Threshold
CFX96™ Real-time PCR detection system (Bio-Rad Laboratories, Inc.)	500
QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.)	300,000
AriaDx Real-time PCR System (Agilent Technologies, Inc.)	2,500

Output cycles of amplification (Ct) are expressed as a numeric value between 1 and 36. If the Ct result is "Undetermined" it means that no signal has been detected above the preset threshold value.

For the interpretation of the result, refer to the "Interpretation table" on the next page.

[1] "ROX" is often selected as default passive reference. If template files are not used, remember to deselect any passive reference.

[2] To obtain precise genotyping of HPV types present in the specimen, set up ramp increment temperature <0.2°C (optimal increment is 0.1°C).

Interpretation table

Ct HR HPV PCR Mix	Ct ß-globin PCR Mix	Tm HR HPV PCR Mix	Tm ß-globin PCR Mix	Test status	Result	Interpretation	Suggested action
Numerical value	Numerical value	In the range of the "Genotyping table"	In the range of the "Genotyping table"	Valid	Detected HPV	Positive to one or more high-risk HPV types - possible genotyping	Genotype the sample using the "Genotyping Table"
Undetermined	Numerical value < 30	-	In or out the range of the "Genotyping table"	Valid	Undetected HPV	Negative	-
Numerical value	Undetermined	In the range of the "Genotyping table"	-	Valid	Detected HPV – Undetermined genotype(s)	Positive to one or more high-risk HPV types - genotyping not possible	See "Troubleshooting"
Numerical value	Numerical value	In the range of the "Genotyping table"	Out-of the range of the "Genotyping table	Valid	Detected HPV – Undetermined genotype(s)	Positive to one or more high-risk HPV types - genotyping not possible	See "Troubleshooting"
Numerical value	Numerical value	Out-of the range of the "Genotyping table"	In the range of the "Genotyping table"	Valid	Undetected HPV	Negative	See "Troubleshooting"
Numerical value	Numerical value	Out-of the range of the "Genotyping table	Out-of the range of the "Genotyping table	Invalid	Undetermined HPV	-	See "Troubleshooting"
Numerical value	Undetermined	Out-of the range of the "Genotyping table	-	Invalid	Undetermined HPV	-	See "Troubleshooting"
Undetermined	Numerical value > 30 or undetermined	-	Not calculated - In or out-of the range of the "Genotyping table"	Invalid	Undetermined HPV)	-	See "Troubleshooting"

Each sample resulting valid and positive for the presence of one or more high-risk HPV types, can be further analyzed to determine specifically which high-risk HPV type(s) is present.

The HPV Selfy HR assay allows to discriminate 14 high-risk HPV types, i.e.: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 by means of analysis of melting temperature (Tm) of the amplified DNA analyte. In the HPV Selfy HR assay, each one of the high-risk HPV types is characterized by a specific Tm interval.

Co-infections of two or more high-risk HPV types whose melting peaks are adjacent, in some cases can originate a single melting peak with an intermediate Tm value between those of the individual high-risk HPV types.

The Tm can be influenced by some factors relating to the biological sample, mainly related to the buffer used in the isolation method, as well as by the PCR instrument. It is advisable to check that the Tm signals originating in the Positive Control correspond to those indicated in the "Genotyping table" on the next page.

Genotyping table

HR HPV PCR Mix								
Instrument								
HPV type	QuantStudio™ 5 Melting Temperature °C		HPV type	AriaDx Melting Temperature °C		HPV type	CFX96™ Melting Temperature °C	
	from	to		from	to		from	to
HPV68	72.00	73.20	HPV68	72.40	73.80	HPV68	72.00	73.30
HPV59	73.30	74.10	HPV59	74.00	74.60	HPV59	73.40	73.60
HPV66	74.50	75.30	HPV66	74.80	75.60	HPV66	73.80	75.30
HPV16	76.00	76.95	HPV16	76.20	77.40	HPV16	75.40	76.80
HPV35	77.00	78.00	HPV35	77.60	78.40	HPV35	76.90	77.40
HPV31	78.20	79.45	HPV31	78.80	79.60	HPV31	77.80	78.90
HPV56	79.50	80.00	HPV56	79.80	80.20	HPV56	79.00	79.70
HPV52	80.05	80.40	HPV52	80.40	80.60	HPV52	79.80	80.00
HPV39	80.60	80.90	HPV39	80.80	81.20	HPV39	80.10	80.30
HPV58	81.20	81.80	HPV58	81.40	82.00	HPV58	80.60	81.00
HPV51	81.85	82.40	HPV51	82.20	82.60	HPV51	81.40	81.60
HPV45	82.50	83.95	HPV45	82.80	83.40	HPV45	81.80	82.80
HPV33	84.60	85.30	HPV33	84.60	85.40	HPV33	83.60	84.80
HPV18	85.60	87.00	HPV18	85.60	87.00	HPV18	84.90	87.00
ß-globin PCR Mix								
Instrument								
Target	QuantStudio™ 5 Melting Temperature °C		Target	AriaDx Melting Temperature °C		Target	CFX96™ Melting Temperature °C	
	from	to		from	to		from	to
ß-globin	75.50	76.80	ß-globin	76.40	77.80	ß-globin	75.00	77.00

F. Quality control

To validate the test results, it is necessary to verify the validity of the PCR run (analysis). For this purpose, a Negative Control and a Positive Control are required for each PCR amplification run, for both the HR HPV PCR Mix and the ß-globin PCR Mix. Negative Control is used to check that no component has been contaminated with nucleic acids during the preparation of the amplification reactions. Positive Control allows to evaluate the assay performance. The analysis is considered valid when both the following conditions are met:

- Positive Control HPV is characterized by amplification curves in the HR HPV PCR Mix and the ß-globin PCR Mix.
- Negative Control is characterized by no amplification curves neither in the HR HPV PCR Mix, nor in the ß-globin PCR Mix.

For a correct genotyping analysis, it is necessary to detect at least one of the three melting peaks of the Positive Control (tested with HR HPV PCR Mix) and the melting peak of the Positive Control (tested with ß-globin PCR Mix), within the melting temperature ranges indicated below:

PCR Mix	Target	Melting Temperature Range °C					
		QuantStudio™ 5		AriaDx		CFX96™	
		from	to	from	to	from	to
HR HPV PCR Mix	HPV68	72.00	73.20	72.40	73.80	72.00	73.30
	HPV56	79.50	80.00	79.80	80.20	79.00	79.70
	HPV18	85.60	87.00	85.60	87.00	84.90	87.00
ß-globin PCR Mix	ß-globin	75.50	76.80	76.40	77.80	75.00	77.00

If an amplification signal exceeding the threshold value for HR HPV PCR Mix or for ß-globin PCR Mix is detected in the Negative Control, the plate is invalidated, and the test must be repeated after eliminating the contamination source. Clean the PCR sample preparation area and repeat the test with a new kit. Ensure that instrument parameters are correctly set.

If anomalies in the amplification of Positive Control are observed, the plate is invalidated, and it has to be repeated. In this case contact the supplier of the product.

If anomalies in the melting curve of Positive Control are observed, the plate valid, but genotyping could not be reliable. In this case contact the supplier of the product.

Troubleshooting

Sample type	Issue / Error	Possible cause	Possible solution
Positive Control	Invalid Positive Control: no amplification curves	Pipetting error.	Take care when dispensing reagents into the microplate wells.
		PCR mix setup error.	Verify to have executed correctly the instructions described in the paragraph "Preparation of amplification PCR Mix".
		Inadequate storage of reagents.	Use a new aliquot of reagents or a new kit.
		DNase presence.	Use DNAse-free consumables.
		PCR failure.	Ensure that instrument's parameters are correct.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
Negative Control	Invalid Negative Control: presence of amplification curves	Local contamination.	Clean PCR preparation area. Ensure that adequate Personal Protection Equipment are used to reduce contamination risk.
		Reagent contamination.	Use a new aliquot of contaminated reagent(s).
		Inadequate storage of reagents.	Use a new aliquot of reagents or a new kit.
		Pipetting error.	Always change tip between samples. Take care when dispensing reagents into the microplate wells.
		PCR mix setup error.	Verify to have executed correctly the instructions described in the paragraph "Preparation of amplification PCR Mix".
		Plate sealing error.	Take care when sealing the plate and follow the manufacturer's instructions.
Biological sample	Inconclusive genotyping - Invalid test: Out-of the range of the "Genotyping table"	Inadequate sample.	Verify sample compatibility and adequacy.
		Inadequate sample collection, storage or transport.	Repeat DNA isolation or sample collection.
		Inadequate DNA isolation. - Chemical contamination.	Verify DNA isolation compatibility. Repeat DNA isolation.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
	Invalid test: no amplification curve in the HR HPV PCR Mix and no amplification curve in the ß-globin PCR Mix or amplification curve with Ct > 30	Inadequate sample.	Verify sample compatibility and adequacy.
		Inadequate sample collection, storage or transport.	Repeat DNA isolation or sample collection.
		Inadequate DNA isolation.	Verify DNA isolation compatibility. Repeat DNA isolation.
		PCR failure.	Ensure that instrument's parameters are correct.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
		PCR inhibitors presence.	Try to dilute isolated DNA 1:5. Repeat DNA isolation or sample collection.

Procedure for ELITe InGenius®

The procedure to use HPV Selfy HR with the ELITe InGenius® system consists of three steps:

A. verification of the system readiness.

B. Setup of the session.

C. Review and approval of results.

A. Verification of the system readiness

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

- switch on the ELITe InGenius® and select the login mode "CLOSED", verify that the amplification controls - Positive Control HPV and Negative Control - were run in association both HR HPV PCR Mix and ß -globin PCR Mix, with the amplification reagent lot to be used and that they are approved and in the valid (Status). If there are not amplification controls approved or valid, run them using the dedicated Assay Protocols reported in section "Materials required but not provided" at bullet point "E", 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 HPV Selfy HR, the ELITe InGenius® instrument and the cited matrices.

The Assay Protocols available for sample testing with the HPV Selfy HR product is described in the table below.

Assay Protocols for HPV Selfy HR			
Name	Matrix/Eluate	Report	Characteristics
Ulisse HPV Mix_CYT_200_200_00	Liquid based cervical cytology specimen	Positive/ Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix: HR HPV PCR Mix PCR Mix volume: 45 µL Sample PCR input volume: 5 µL
Ulisse HPV Mix_CS_200_200_00	Cervical/Vaginal swabs	Positive/ Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix: HR HPV PCR Mix PCR Mix volume: 45 µL Sample PCR input volume: 5 µL
Ulisse Bglobin Mix_CYT_200_200_00	Liquid based cervical cytology specimen	Valid/ Not valid	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix: ß-globin PCR Mix PCR Mix volume: 45 µL Sample PCR input volume: 5 µL

Name	Matrix/Eluate	Report	Characteristics
Ulisse Bglobin Mix_CS_200_200_00	Cervical/Vaginal swabs	Valid/ Not valid	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix: ß-globin PCR Mix PCR Mix volume: 45 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.

B. Setup of the session

The HPV Selfy HR product, in association with the ELITe InGenius® system, can be used 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 Protocols available on the instrument and are automatically recalled when the Assay Protocols are selected.

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

The main steps for the setup of the three types of runs are described here below:

a. Integrated run

To setup an integrated run starting from the primary sample, carry out the following steps as per the Graphic User Interface (GUI):

- thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.
- Prepare two 2 mL Micro Tubes (Sarstedt) which will contain the HR HPV PCR Mix and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.
- Prepare the HR HPV PCR Mix and the ß-globin PCR Mix according to the following tables:

HR HPV PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
HR HPV Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-
ß-globin PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
ß-globin Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-

- At the end, vortex the mix and briefly spin them, avoiding the formation of bubbles.
- Select "Perform Run" from the "Home" screen.
- Ensure that the "Extraction Input Volume" is 200 µL and the "Extraction Elute Volume" is 200 µL.
- If needed, transfer 200 µL of the sample from primary tube to an ELITe InGenius® "Extraction Tube".
- For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
- Select the Assay Protocol to be used in the "Assay" column (i.e. "Ulisse HPV Mix_CYT_200_200_00").
- Ensure that the "Protocol" displayed is: "Extract + PCR".
- Select the sample loading position in the "Sample Position" column:
  - if a primary tube is used, select "Primary Tube";
  - if a secondary tube is used, select "Extraction Tube".
- Click "Next" to continue the setup.
- Load the freshly prepared HPV PCR Mix and ß -globin PCR Mix and on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of HPV Selfy HR kit. Click "Next" button to continue the setup.
- Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
- 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.
- Close the instrument door.
- Press "Start" to start the run.
- After process completion, the ELITe InGenius® system allows users to view, approve, store the results and to print and save the report.

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

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

b. Amplification run

To set up the amplification run starting from an already eluted sample, carry out the following steps as per GUI:

- thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.
- Prepare two 2 mL Micro Tubes (Sarstedt) which will contain the HR HPV PCR Mix and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.
- Prepare the HR HPV PCR Mix and the ß-globin PCR Mix according to the following tables:

HR HPV PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
HR HPV Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-
ß-globin PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
ß-globin Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-

- At the end, vortex the mix and briefly spin them, avoiding the formation of bubbles.
- Select "Perform Run" from the "Home" screen.
- Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 200 µL.
- For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
- Select the Assay Protocol to be used in the "Assay" column (i.e. "Ulisse HPV Mix_CYT_200_200_00"). Select "PCR Only" in the "Protocol" column.
- Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Click "Next" to continue the setup.
- Load the freshly prepared HPV PCR Mix and ß -globin PCR Mix and on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of HPV Selfy HR kit. Click "Next" button to continue the setup.
- Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
- Load the "PCR Cassette" and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.
- Close the instrument door.
- Press "Start" to start the run.
- After process completion, the ELITe InGenius® system allows users to view, approve, store the results and to print and save the report.

Note: at the end of the run the remaining Extracted Sample must be removed from the instrument, capped 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

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

- thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.
- Prepare two 2 mL Micro Tubes (Sarstedt) which will contain the HR HPV PCR Mix and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.
- Prepare the HR HPV PCR Mix and the ß-globin PCR Mix according to the following tables:

HR HPV PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
HR HPV Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-
ß-globin PCR Mix			
Reagent	Volume per sample or control	Volume for n <= 4	Volume for 4 < n <= 6
Reaction Mix (DNA)	30.0 µL	30.0 x (n + 1) µL	30.0 x (n + 2) µL
$MgCl_2$ 25mM solution	1.5 µL	1.5 x (n + 1) µL	1.5 x (n + 2) µL
ß-globin Mix	13.5 µL	13.5 x (n + 1) µL	13.5 x (n + 2) µL
Total volume	45.0 µL	-	-

- At the end, vortex the PCR mix and briefly spin them, avoiding the formation of bubbles.
- Thaw the Positive Control HPV tube for the session. Each tube is sufficient for 2 sessions. Mix gently, spin down the content for 5 seconds. Transfer at least 50 µL of Positive Control HPV to 2 "Elution tubes", provided with the "ELITe InGenius® SP 200 Consumable Set".
- Thaw the Negative Control tube for the session. Each tube is sufficient for 2 sessions. Mix gently, spin down the content for 5 seconds. Transfer at least 50 µL of Negative Control to 2 "Elution tubes", provided with the "ELITe InGenius® SP 200 Consumable Set".
- Select "Perform Run" from the "Home" screen.
- In the Track of interest, select the Assay Protocol to be used in the "Assay" column.
- For the Track of positive control to be amplified with HR HPV PCR Mix, select the Assay Protocol "Ulisse HPV Mix_PC_00" in the "Assay" column and fill in the lot number and expiry date of Positive Control HPV.
- For the Track of negative control to be amplified with HR HPV PCR Mix, select the Assay Protocol "Ulisse HPV Mix_NC_00" in the "Assay" column and fill in the lot number and expiry date of the Negative Control.
- For the Track of positive control to be amplified with ß-globin PCR Mix, select the Assay Protocol "Assay_Ulisse Bglobin Mix_PC_00" in the "Assay" column and fill in the lot number and expiry date of Positive Control HPV.
- For the Track of negative control to be amplified with ß-globin PCR Mix, select the Assay Protocol "Assay_Ulisse Bglobin Mix_NC_00" in the "Assay" column and fill in the lot number and expiry date of the Negative Control.

- Click “Next” to continue the setup.
- Load the freshly prepared HR HPV PCR Mix and ß -globin PCR Mix and on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of HPV Selfy HR kit. Click “Next” button to continue the setup.
- Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
- Load the “PCR Cassettes”, the Positive Control HPV Elution tubes and the Negative Control Elution tubes following the GUI instruction. Click “Next” to continue the setup.
- Close the instrument door.
- 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, identified and stored at -20 °C. Avoid spilling the Positive Control. The remaining Negative Control must be disposed.

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

C. 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 ELITe InGenius® instrument user's manual for more details.

Note: 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 ELITe InGenius® instrument user's manual for more details.

The ELITe InGenius® system generates the results with the product HPV Selfy HR through the following procedure:

a) validation of amplification Positive Control and Negative Control results.
b) Validation of sample results.
c) Sample result reporting.

a. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the amplicons of the 3 high-risk HPV types (HR HPV PCR Mix) and for the IC (ß-globin PCR Mix) in the Positive Control HPV and Negative Control amplification reaction are analyzed and interpreted by the ELITe InGenius® software with the parameters included in the Assay Protocols "Ulisse HPV Mix_PC_00", "Assay_Ulisse Bglobin Mix_PC_00", "Ulisse HPV Mix_NC_00" and "Assay_Ulisse Bglobin Mix_NC_00".

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

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

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

Note: When approving the result of the amplification of the Positive Control, to verify the reliability of the genotyping it is necessary to check the graphs of the melting curve of the mixes in the work session report. The melting temperatures (Tm) to be observed in the Positive Control are listed in the table called "Positive Control - Melting temperatures observed on ELITe InGenius®" shown below. For a reliable genotyping at least one of the three melting peaks of the Positive Control tested with the HR HPV PCR Mix and the melting peak of the Positive Control tested with the ß-globin PCR Mix must be detected.

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

Note: when the Positive Control and/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.

Positive Control - Melting temperatures observed on ELITe InGenius®

PCR Mix	Positive Control target	Melting temperature range °C	
		from	to
HR HPV PCR Mix	HPV68	72.0	73.3
	HPV56	79.4	80.1
	HPV18	85.6	87.0
ß-globin PCR Mix	ß-globin	75.2	77.4

b. Validation of Sample results

The fluorescence signals emitted by the amplicons of the 14 high-risk HPV types (HR HPV PCR Mix) and for the IC (ß-globin PCR Mix) in the sample amplification reactions are analyzed and interpreted by the instrument software with the parameters included in the Assay Protocol.

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 "Ulisse HPV Mix_PC_00"	APPROVED
HR HPV Positive Control "Assay_Ulisse Bglobin Mix_PC_00"	APPROVED
2) Negative Control	**Status**
Negative Control "Ulisse HPV Mix_NC_00"	APPROVED
Negative Control "Assay_Ulisse Bglobin Mix_NC_00"	APPROVED

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

The possible result messages of a sample are listed the table below.

Result of sample run with HR HPV PCR Mix	Interpretation
HPV:DNA Detected Check Tm result	One or more high-risk HPV type(s) is detected with Ct calculated. Check Tm results for genotype.
HPV:Check Bglobin Mix result	Sample has a Ct value Undetermined and a Tm value calculated. Sample is HPV-negative. Sample has a Ct value Undetermined and a Tm value not calculated. Sample is HPV-negative. Sample validity depends, in both cases, on ß-globin Ct value and Tm value obtained.
Result of sample run with ß-globin PCR Mix	**Interpretation**
Bglobin:DNA Detected Tm within range	Sample has a ß-globin Ct value lower than 30, a ß-globin Tm value within the acceptance range. Sample is valid.
Bglobin:DNA Detected Tm out of range-Retest Sample	Sample has a ß-globin Ct value lower than 30 but a Tm value out of the acceptance range. Sample is invalid.
Invalid – retest the sample	Sample has a ß-globin Ct value higher than 30. Sample is invalid.

HPV positive samples are reported as "HPV:DNA Detected Check Tm result" by the ELITe InGenius® software. In this case, genotyping must be evaluated after the run, manually checking Tm values obtained.

If the HPV Ct value is calculated and the sample is reported as "HPV:DNA Detected Check Tm result" by the ELITe InGenius® software, but Tm values are not calculated, the dissociation curve analysis was not efficiently carried out due to problems with sample (inhibitors carry-over in the eluate or presence of other interfering SNPs), which may cause incorrect results. In this case, the sample have to be retested.

Samples with Ct value Undetermined for HPV target, with and without a calculated Tm, are reported as "HPV:Check Bglobin Mix result" by the ELITe InGenius® software. In this case, the sample is considered HPV-negative. Sample validity depends on ß-globin Ct value and Tm value obtained. In this case, if the respective result obtained with ß-globin PCR Mix is reported as "Bglobin: DNA Detected Tm within range" by the ELITe InGenius® software, sample is considered a valid negative.

Samples results related to ß-globin target reported as "Invalid - Retest Sample" by the ELITe InGenius® software are not suitable for result interpretation. In this case, the human genomic DNA of the sample was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction, inhibitors carry-over in the eluate or DNA quantity in the sample not sufficient), which may cause incorrect results. When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in "PCR Only" mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using "Extract + PCR" mode.

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

Note: when approving the assay results, always verify the instrument outcome by checking the melting curve plots in the work session report. The melting temperatures (Tm) of each HR HPV genotype in analysis have to correspond to the peaks showed in the melting curve plots (see table below).

Co-infections of two or more HPV types whose melting peaks are adjacent, in some cases can originate a single melting peak with an intermediate Tm value between those of the individual HPV types.

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".

Genotyping table for ELITe InGenius®

HR HPV PCR Mix		
HPV Type	Melting Temperature °C	
	from	to
HPV68	72.0	73.3
HPV59	73.4	74.0
HPV66	74.2	75.2
HPV16	76.0	77.0
HPV35	77.3	78.3
HPV31	78.5	79.2
HPV56	79.4	80.1
HPV52	80.2	80.5
HPV39	80.6	81.0
HPV58	81.1	81.7
HPV51	81.9	82.3
HPV45	82.4	83.0
HPV33	84.8	85.4
HPV18	85.6	87.0
ß-globin PCR Mix		
Target	Melting Temperature °C	
	from	to
ß-globin	75.2	77.4

c. *Sample result reporting*

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

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

The "Track Report" shows the details of a work session by selected Track.

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

Troubleshooting for ELITe InGenius®

Sample type	Issue / Error	Possible cause	Solution
Positive Control	Invalid Positive Control reaction	Instrument setting error.	Check the position of HR HPV PCR Mix and positive control. Check the volumes of HR HPV PCR Mix and positive control.
		Positive control degradation.	Use a new aliquot of positive control.
		PCR Mix degradation.	Use a new aliquot of HR HPV PCR Mix.
		Instrument error.	Contact ELITechGroup Technical Service.
Negative Control	Invalid Negative Control reaction	Instrument setting error.	Check the position of HR HPV PCR Mix and negative control. Check the volumes of HR HPV PCR Mix and negative control.
		Contamination of the negative control	Use a new aliquot of molecular-biology grade water.
		Contamination of the PCR Mix.	Use a new aliquot of HR HPV PCR Mix.
		Contamination of the Extraction Area, of Racks or of Inventory Block	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
		Instrument error.	Contact ELITechGroup Technical Service.
Biological sample	Invalid or Inconclusive Sample reaction	Instrument setting error.	Check the position of HR HPV PCR Mix and sample. Check the volumes of HR HPV PCR Mix and sample.
		PCR Mix degradation.	Use a new aliquot of HR HPV PCR Mix.
		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.
		DNA quantity not sufficient in the sample.	Repeat the extraction and amplification with a new aliquot of the sample in a “Extract + PCR” session.
		Instrument error.	Contact ELITechGroup Technical Service.
N.A.	Error 30103	Too high concentration of the target in the sample.	Repeat the amplification reaction of the sample with a 1:10 dilution of the eluted sample in molecular-biology grade water in a session in "PCR Only" mode.
N.A.	TH Error, SDM error, Ct error	Sample with anomalous plot shape.	Repeat the amplification reaction of the sample with a 1:10 dilution of the eluted sample in molecular-biology grade water in a session in "PCR Only" mode.

Procedure for HYRIS bCUBE™

The procedure to use HPV Selfy HR with HYRIS bCUBE™ consists of nine steps:

A. nucleic acid isolation.
B. Recipe setup.
C. bAPP™ analysis setup.
D. Cartridge setup.
E. Preparation of amplification PCR mixes.
F. PCR plate assembly.
G. Analysis start.
H. Interpretation of tests results.
I. Quality control.

A. Nucleic acid isolation

Various manufacturers offer nucleic acid isolation kits. Use the right amount of sample according to the protocol in use. The following isolation kits have been validated for use with HPV Selfy HR.

a. Preparation of liquid based cervical cytology specimens

Before pretreatment with Ulisse Faster DNA or DNA extraction, liquid based cervical cytology specimens stored in Thin Prep® must be prepared as indicated hereby:

- vortex the Thin Prep® vial for at least 30 seconds to homogenize the sample.
- Transfer 1.5 mL of liquid based cervical cytology specimen from the original Thin Prep® vial into a 1.5 mL Eppendorf tube.
- Centrifuge the tube at 9,000 g for 3 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet. Excessive leftover of Thin Prep® solution could cause inhibition of the following PCR reaction.
- Add 1 mL of 1x Phosphate Buffered Saline solution (PBS) to the cell pellet and place the tube on the vortex for at least 30 seconds.
- Centrifuge the tube at 9,000 g for 3 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet.
- Resuspend in 80 µL of molecular-biology grade water.

The prepared samples can be now pretreated with Ulisse Faster DNA or extracted with DNA extraction kits following manufacturer's instructions.

b. Preparation of cervical and vaginal swab specimens

Before pretreatment with Ulisse Faster DNA or DNA extraction, vaginal or cervical swab specimens must be resuspended as indicated hereby:

- use a pipette with a disposable tip to transfer 2 mL of molecular-biology grade water into the 5 mL tube.
- Immerse the swab in the water with a series of rapid vertical movements; subsequently and without being immersed, the plug must be rotated by pressing it against the walls of the tube in order to facilitate the release of as much material as possible.
- Make the suspension homogeneous by vortexing it for 10-20 seconds so that no precipitate is visible.
- The prepared samples can be now pretreated with Ulisse Faster DNA or extracted with DNA extraction kits following manufacturer's instructions.

c. *Compatible isolation kits*

The following isolation kits have been validated for use with HPV Selfy HR:

- QIAamp® DNA Mini Kit (Qiagen, Inc.; code #51304); elute in molecular-biology grade water.
- Reliaprep™ Blood gDNA Miniprep System (Promega, Corp.; code #A5082).
- Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014).

B. Recipe setup

1. Open the browser and search for https://bapp2.HYRIS.net.
2. Login to the HYRIS bAPP™ selecting the country (i.e. "Europe") and inserting username and password.
3. Click on the menu "Recipes".
4. Click on "New Recipe".
5. Complete all the field to setup the recipe with the information reported below.
6. Name the recipe with an unique name to associate the recipe to the kit in use, for instance "**Ulisse BioMed – UBM0013 – HPV Selfy HR".**

Parameters for the recipe setup:

Detection channel	Yes/No	Intensity	Exposition
FAM	Yes	100%	50%

Well type	
Name	**Description**
HR HPV PCR Mix	14 HR HPV
β-globin PCR Mix	β-globin

Sample type	
Name	**Description**
PCHR	Positive Control
NC	Negative Control
Sample	Sample

Phase	Phase		Step bAPP™ recipe	Time	Temperature	Data collection
PCR	Polymerase activation		Constant temperature step	30 sec	98.0 °C	-
	Denaturation	repeat 36 cycles	PCR amplification	5 sec	98.0 °C	-
	Annealing			10 sec	61.5 °C	-
	Extension			5 sec	72.0 °C	yes
Melting curve	Denaturation		Constant temperature step	15 sec	95.0 °C	-
	Start melting		Melting	60 sec	60.0 °C	-
	Optimal ramp increment / Soak time[3]			0.033°C / sec		yes
	End melting			1 sec	95.0 °C	-

For setting the cartridge layout and the assisted interpretation of results, refer to the HYRIS bAPP™ instruction manual.

[3] To obtain precise genotyping of HPV types present in the specimen, set up ramp increment temperature <0.2°C (optimal increment is 0.1°C).

C. bAPP™ analysis setup

1. Log in to HYRIS bAPP™ (https://bapp2.HYRIS.net) with username and password.
2. Initialize a new analysis from the Analysis menu by clicking on "Create analysis".
3. Fill in the "General Information" page:
   - name of the analysis.
   - Description (lot of the kit).
4. Select Use Custom Recipe.
5. Select the recipe from the drop-down menu: Ulisse BioMed – UBM0013 – HPV Selfy HR.
6. Click on "Continue" to go on.

D. Cartridge setup

1. Select the cartridge type between 16- and 36-well layouts.
2. For each sample, scan or type the sample ID in the "Sample Name" field.
3. When finished entering the IDs, press "Continue".
4. Check the position of the samples on the cartridge layout.
5. Save the initialized analysis for later by dragging the selector above the "Finish" button.
6. Click "Save".

Note: it is advisable to identify with the red color the wells reserved for the Positive Controls (one for each mix), and with the green color the wells reserved for the Negative Controls (one for each mix).

Note: This kit is compatible with both 16-well and 36-well cartridges.

E. Preparation of amplification PCR mix

Thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.

Prepare two 1.5 mL polypropylene capped tubes which will contain the HR HPV PCR Mix and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.

Prepare the HR HPV PCR Mix and the ß-globin PCR Mix: for each session, combine the following components sufficient for the number of samples to be tested plus one Positive Control and one Negative Control. All volumes include 10% overage for pipette error.

HR HPV PCR Mix		
Reagent	Volume per sample or control	Volume for "n" samples plus 2 controls
Reaction Mix (DNA)	12.00 µL	13.20 x (n + 2) µL
$MgCl_2$ 25mM solution	0.60 µL	0.66 x (n + 2) µL
HR HPV Mix	5.40 µL	5.94 x (n + 2) µL
Total Volume	18.00 µL	-
ß-globin PCR Mix		
Reagent	Volume per sample or control	Volume for "n" samples plus 2 controls
Reaction Mix (DNA)	12.00 µL	13.20 x (n + 2) µL
$MgCl_2$ 25mM solution	0.60 µL	0.66 x (n + 2) µL
ß-globin Mix	5.40 µL	5.94 x (n + 2) µL
Total Volume	18.00 µL	-

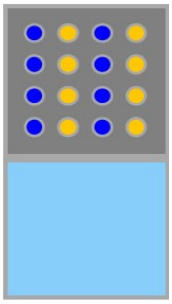
At the end, vortex the mix and briefly spin them, avoiding the formation of bubbles.

F. PCR plate assembly

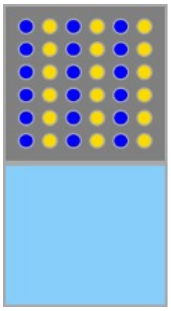
Load 18 µL of HR HPV PCR Mix, and 18 µL of ß-globin PCR Mix for each sample in two separate wells.

Load twice 2 µL of each biological sample (S1, S2, S3, etc), of Positive Control (PC) and of Negative Control (NC): once in the HR HPV PCR Mix-loaded well and once in the ß-globin PCR Mix-loaded well, as indicated in the figure below, where HR HPV PCR mix is indicated in blue and ß-globin PCR Mix is indicated in yellow (Figure A for 16 wells cartridge, Figure B for 36 wells cartridge).

A



B



Seal the PCR plate using adequate adhesive seals following manufacturer's instructions.

Insert immediately the cartridge in the HYRIS bCUBE™ and start the analysis.

Note: Do not use any adhesive film not included in the packaging of the cartridges supplied by HYRIS, S.r.l.. Do not insert the cartridge inside the HYRIS bCUBE™ without having first sealed it with the aluminum adhesive film.

G. Analysis start

1. Open the "Analysis" menu of the HYRIS bAPP™.
2. Select the initialized analysis.
3. Click on the "Launch Analysis" button.
4. Select the serial code (S/N) of the bCUBE™.
5. Click on the "GO" button.
6. At the end of the analysis remove the cartridge from the bCUBE™ and dispose of it in accordance with local and state regulations.
7. At the end of the analysis, the results are displayed on the "PCR" screen of the Hyris bAPP™.

The entire procedure is also reported in the Practical User Guide, available on HYRIS bAPP™ and on the HYRIS Help Center https://support.HYRIS.net.

H. Interpretation of tests results

The recorded values of the fluorescence in the amplification reactions must be analyzed by the instrument software. Data analysis is performed with the instrument system software, and according to manufacturer's instruction.

At the end of the run the results are shown in the HYRIS bAPP™ "PCR" screen (run amplification curves screen), which shows the results for each sample and its cycle threshold (Ct).

The fluorescence values allow to determine the threshold cycle the cycle in which the fluorescence has reached the threshold value. Before starting the analysis, set the threshold as follows, referring to the HYRIS bAPP™ instruction manual.

PCR Mix	Threshold
HR HPV PCR Mix	10,000
ß-globin PCR Mix	10,000

Output cycles of amplification (Ct) are expressed as a numeric value between 1 and 36. If the Ct result is "Undetermined" it means that no signal has been detected above the preset threshold value. For the interpretation of the result, refer to the "Interpretation Table for HYRIS bCUBE™" on the next page.

Interpretation table for HYRIS bCUBE™

Ct HR HPV PCR Mix	Ct ß-globin PCR Mix	Tm HR HPV PCR Mix	Tm ß-globin PCR Mix	Test status	Result	Interpretation	Suggested action
Numerical value	Numerical value	In the range of the "Genotyping table"	In the range of the "Genotyping table"	Valid	Detected HPV	Positive to one or more high-risk HPV types; possible genotyping	Genotype the sample using the "Genotyping Table for HYRIS bCUBE™"
Undetermined	Numerical value < 30	-	In or out the range of the "Genotyping table"	Valid	Undetected HPV	Negative	-
Numerical value	Undetermined	In the range of the "Genotyping table"	-	Valid	Detected HPV – Undetermined genotype(s)	Positive to one or more high-risk HPV types; not possible genotyping	See "Troubleshooting for HYRIS bCUBE™"
Numerical value	Numerical value	In the range of the "Genotyping table"	Out-of the range of the "Genotyping table	Valid	Detected HPV – Undetermined genotype(s)	Positive to one or more high-risk HPV types; not possible genotyping	See "Troubleshooting for HYRIS bCUBE™"
Numerical value	Numerical value	Out-of the range of the "Genotyping table"	In the range of the "Genotyping table"	Valid	Undetected HPV	Negative	See "Troubleshooting for HYRIS bCUBE™"
Numerical value	Numerical value	Out-of the range of the "Genotyping table	Out-of the range of the "Genotyping table	Invalid	Undetermined HPV	-	See "Troubleshooting for HYRIS bCUBE™"
Numerical value	Undetermined	Out-of the range of the "Genotyping table	-	Invalid	Undetermined HPV	-	See "Troubleshooting for HYRIS bCUBE™"
Undetermined	Numerical value > 30 or undetermined	-	Not calculated - In or out-of the range of the "Genotyping table"	Invalid	Undetermined HPV	-	See "Troubleshooting for HYRIS bCUBE™"

It is possible to set up assisted interpretation of results to have the system interpret the Ct values of samples and controls, as well as melting curves, and determine an interpreted result such as:

- DETECTED HPV;
- UNDETECTED HPV.

To set up assisted interpretation and interpretive labels refer to the "Interpretation table for HYRIS bCUBE™".

The procedure for setting up assisted interpretation is available in the HYRIS bAPP™ manual, at https://support.hyris.net or by contacting your HYRIS specialist at support@hyris.net.

Each sample resulting valid and positive for the presence of one or more high-risk HPV types, can be further analyzed to determine specifically which high-risk HPV type(s) is present.

The HPV Selfy HR assay allows to discriminate 14 high-risk HPV types, i.e.: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 by means of analysis of melting temperature (Tm) of the amplified DNA analyte. In the HPV Selfy HR assay, each one of the high-risk HPV types is characterized by a specific Tm interval.

Co-infections of two or more high-risk HPV types whose melting peaks are adjacent, in some cases can originate a single melting peak with an intermediate Tm value between those of the individual high-risk HPV types.

The Tm can be influenced by some factors relating to the biological sample, mainly related to the buffer used in the isolation method, as well as by the PCR instrument. It is advisable to check that the Tm signals

originating in the positive control correspond to those indicated in the "Genotyping table for HYRIS bCUBE™" on the next page.

Genotype assisted interpretation and analysis can also be set up for each sample in the assisted interpretation of the HYRIS bAPP™. The numerical values of Tm shown in the "Genotyping table for HYRIS bCUBE™" can be associated with a precise genotype and the result can be shown in the "RESULTS" section of the HYRIS bAPP™ or in a PDF report.

Genotyping table for HYRIS bCUBE™

HR HPV PCR Mix		
HPV type	Melting Temperature °C	
	from	to
HPV68	71.80	73.19
HPV59	73.20	73.90
HPV66	74.00	75.00
HPV16	75.20	77.00
HPV35	77.20	78.00
HPV31	78.20	79.10
HPV56	79.11	79.75
HPV52	79.76	80.09
HPV39	80.16	80.60
HPV58	80.80	81.30
HPV51	81.50	81.80
HPV45	82.00	83.00
HPV33	84.00	84.99
HPV18	85.00	86.50
ß-globin PCR Mix		
Target	Melting Temperature °C	
	from	to
ß-globin	74.00	77.00

I. Quality control

To validate the test results. it is necessary to verify the validity of the PCR run (analysis). For this purpose, a Negative Control and a Positive Control are required for each PCR amplification run, for both the HR HPV PCR Mix and the ß-globin PCR Mix. Negative Control is used to check that no component has been contaminated with nucleic acids during the preparation of the amplification reactions. Positive Control allows to evaluate the assay performance. The analysis is considered valid when both the following conditions are met:

- Positive Control HPV is characterized by amplification curves in the HR HPV PCR Mix and the ß-globin PCR Mix.
- Negative Control is characterized by no amplification curves neither in the HR HPV PCR Mix, nor in the ß-globin PCR Mix.

For a correct genotyping analysis, it is necessary to detect one of the three melting peaks of the Positive Control (tested with HR HPV PCR Mix) and the melting peak of the Positive Control (tested with ß-globin PCR Mix), within the melting temperature ranges indicated below:

PCR Mix	Positive Control Target	Melting Temperature Range °C	
		from	to
HR HPV PCR Mix	HPV68	71.80	73.19
	HPV56	79.11	79.75
	HPV18	85.00	86.50
ß-globin PCR Mix	ß-globin	74.00	77.00

If an amplification signal exceeding the threshold value for HR HPV PCR Mix or for ß-globin PCR Mix is detected in the Negative Control, the plate is invalidated, and the test must be repeated after eliminating the contamination source. Clean the PCR sample preparation area and repeat the test with a new kit. Ensure that instrument parameters are correctly set.

If anomalies in the amplification of Positive Control are observed, the plate is invalidated, and it has to be repeated. In this case contact the supplier of the product.

If anomalies in the melting curve of Positive Control are observed, the plate valid, but genotyping could not be reliable. In this case contact the supplier of the product.

Troubleshooting for HYRIS bCUBE™

Sample type	Issue / Error	Possible cause	Possible solution
Positive Control	Invalid Positive Control: no amplification curves	Pipetting error.	Take care when dispensing reagents into the microplate wells.
		PCR mix setup error.	Verify to have executed correctly the instructions described in the paragraph "Preparation of amplification PCR Mix".
		Inadequate storage of reagents.	Use a new aliquot of reagents or a new kit.
		DNase presence.	Use DNAse-free consumables.
		PCR failure.	Ensure that instrument's parameters are correct.
		Error in entering the cartridge.	Ensure to have properly entered the cartridge in the bCUBE™ in the right side.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
Negative Control	Invalid Negative Control: presence of amplification curves	Local contamination.	Clean PCR preparation area. Ensure that adequate Personal Protection Equipment are used to reduce contamination risk.
		Reagent contamination.	Use a new aliquot of contaminated reagent(s).
		Inadequate storage of reagents.	Use a new aliquot of reagents or a new kit.
		Pipetting error.	Always change tip between samples. Take care when dispensing reagents into the microplate wells.
		PCR mix setup error.	Verify to have executed correctly the instructions described in the paragraph "Preparation of amplification PCR Mix".
		Plate sealing error.	Take care when sealing the plate and follow manufacturer's instructions.
Biological sample	Inconclusive genotyping - Invalid test: Out-of the range of the "Genotyping table"	Inadequate sample.	Verify sample compatibility and adequacy.
		Inadequate sample collection, storage or transport.	Repeat DNA isolation or sample collection.
		Inadequate DNA isolation. - Chemical contamination.	Verify DNA isolation compatibility. Repeat DNA isolation.
		Error in entering the cartridge.	Ensure to have properly entered the cartridge in the bCUBE™ in the right side.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
	Invalid test: no amplification curve in the HR HPV PCR Mix and no amplification curve in the ß-globin PCR Mix or amplification curve with Ct > 30	Inadequate sample.	Verify sample compatibility and adequacy.
		Inadequate sample collection, storage or transport.	Repeat DNA isolation or sample collection.
		Inadequate DNA isolation.	Verify DNA isolation compatibility. Repeat DNA isolation.
		PCR failure.	Ensure that instrument's parameters are correct.
		Error in entering the cartridge.	Ensure to have properly entered the cartridge in the bCUBE™ in the right side.
		Bubbles in the PCR reaction.	Repeat the test ensuring to avoid bubbles formation in the well.
		PCR inhibitors presence.	Try to dilute isolated DNA 1:5. Repeat DNA isolation or sample collection.

4. Limits

HPV Selfy HR detects DNA of the 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). This test does not detect DNA of other HPV types. HPV Selfy HR provides a qualitative result.

HPV Selfy HR should only be used with cervical swab, vaginal swab and liquid based cervical cytology specimens. Consult the manufacturer's instructions for technical specifications, limitations, warnings and instructions on the use of the collection devices. The performance of the method has not been evaluated with other types of samples.

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 nucleic acid isolation kits.

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 control 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.

The presence of blood can interfere with HPV Selfy HR.

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 Product performance). In this case the result could be a false negative.

Moreover, test results may be affected by improper specimen collection, technical error, or specimen mix-up, as well as by the presence of interfering substances.

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

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA.

Prevalence of HPV infection in a population may affect performance. Positive predictive value decreases when testing populations with low prevalence or individuals with no risk of infection.

HPV infection is not an indicator of the presence of a high-grade cytological lesion (HSIL) or a precancerous intraepithelial lesion (CIN), nor does it imply that a CIN2 / 3 lesion or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2 / 3 or cancer.

A negative HPV test does not rule out the possibility of developing a high-grade cytological lesion (HSIL) or a precancerous intraepithelial CIN2 / 3 lesion or cancer. A small percentage of such lesions and tumors occur in women who are found to be HPV-negative based on existing technologies.

HPV Selfy HR should be used in conjunction with clinical information from other diagnostic and screening tests, physical medical inspection, and complete medical history, according to appropriate patient management. HPV Selfy HR should not be used as the sole method of diagnosing and treating patients.

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.

HPV Selfy HR has not been evaluated for the management of women with previous cytological or histological abnormalities, hysterectomy, less than 25 years or more than 64 years, postmenopausal or with other risk factors (HIV+, immune-compromised, exposed to Diethylstilbestrol, with previous sexually transmitted diseases).

5. Product performance

All performance characteristics data were determined using manual result interpretation and QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.). Similar performance on CFX96™ Real-time PCR detection system (Bio-Rad Laboratories, Inc.), on AriaDx Real-time PCR System (Agilent Technologies, Inc.), on ELITe InGenius® (ELITechGroup S.p.A.) and on HYRIS bCUBE™ (HYRIS, S.r.l.) has been established by equivalence studies.

Analytical sensitivity

The analytical sensitivity, or Limit of detection (LoD), is defined as the lowest concentration which >95% of the tested samples generate a positive result. LoD of HPV Selfy HR was determined by spiking full genome HPV plasmids at known concentration. The LoD of HPV Selfy HR is 100 copies/reaction for all HPV types, except for HPV 51 (1,000 copies/reaction).

Analytical specificity (cross-reactivity)

The potential cross-reactivity of the HPV Selfy HR assay was evaluated through testing a panel of 17 organisms (bacteria, viruses and fungi), 20 other HPV types (both probable high-risk and low-risk types), and human genomic DNA. No cross-reactivity was observed in these group of pathogens.

Organism	Concentration	Result | Ct
Campylobacter jejuni	10^4 copies / reaction	Negative
Candida albicans	10^4 copies / reaction	Negative
Chlamydia trachomatis	10^4 copies / reaction	Negative
Cytomegalovirus	10^4 copies / reaction	Negative
Gardnerella vaginalis	10^4 copies / reaction	Negative
Herpes Simplex 1	10^4 copies / reaction	Negative
Herpes Simplex 2	10^4 copies / reaction	Negative
HIV-1 (ds0NA gag-env-pol)	10^4 copies / reaction	Negative
Human genomic DNA	10^4 copies / reaction	Negative
HPV6, 11, 26, 40, 41, 42, 43, 44, 53, 54, 55, 61, 64, 67, 69, 70, 71, 72, 73, 82	10^4 copies / reaction	Negative
Mycoplasma genitalium	10^4 copies / reaction	Negative
Mycoplasma hominis	10^4 copies / reaction	Negative
Neisseria flava	10^4 copies / reaction	Negative
Neisseria gonorrhoeae	10^4 copies / reaction	Negative
Neisseria meningiditis	10^4 copies / reaction	Negative
Treponema pallidum	10^4 copies / reaction	Negative
Trichomonas vaginalis	10^4 copies / reaction	Negative
Ureaplasma parvum	10^4 copies / reaction	Negative
Ureaplasma urealytlcum	10^4 copies / reaction	Negative

Interference

HPV Selfy HR uses well established conventional nucleic acid isolation methods and based on our experience with other similar assays, we do not expect interference from common endogenous substances.

Regarding interference of substances in the case HPV Selfy HR is used in a direct PCR mode upon Ulisse Faster DNA pretreatment, the following substances have been investigated for interference. No interference was observed for vaginal douches containing 0.2% hyaluronic acid, up to 50% concentration, whereas blood has an inhibitory power already at 10% concentration. Other interference substances have not been tested.

Analytical reproducibility

The reproducibility of HPV Selfy HR was determined by analyzing full-genome HPV plasmids; each comparison was performed by several operators, each of whom used different PCR machines. The inter-assay coefficient of variation (CV) calculated on the amplification cycles (Ct) is lower than 5%.

Analytical repeatability

The intra-assay Coefficient of Variation (CV) for the Ct value was measured on 10 replicates of different full-genome HPV plasmids diluted to 1,000 copies / reaction, analyzed in the same PCR analysis. All CVs found are lower than 5%.

Clinical Performance

The clinical performance of HPV Selfy HR was evaluated on a library of 98 liquid based cervical cytology specimens from women over 30 years of age and histology >CIN2, and 791 liquid based cervical cytology specimens from women over 30 years of age, with negative cytology and previous negative Pap Test result, all previously tested with Digene HC2 high-risk HPV DNA (Qiagen, Inc.; code #5197-1330). These liquid based cervical cytology specimens were analyzed with HPV Selfy HR after extraction with Reliaprep™ Blood gDNA Miniprep System (Promega, Corp.; code #A5082). The relative sensitivity of HPV Selfy HR compared to the Digene HC2 high-risk HPV DNA (Qiagen, Inc.; code #5197-1330) performed on the same samples is 1.07 (87/98 HPV Selfy HR vs 81/98 HC2), while the relative specificity is 0.92 (680/791 HPV Selfy HR vs 742/791 HC2).

A subset of 144 liquid based cervical cytology specimens (72 positive and 72 negative) were also pretreated with Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014) and then analyzed with HPV Selfy HR; the agreement between the two methods was 100%.

119 self-collected vaginal swabs derived from CIN2+ women and 791 paired self-collected vaginal swabs collected from women with negative cytology and previous negative Pap Test were analyzed with HPV Selfy HR in comparison with HPV Selfy HR executed on paired clinician-collected liquid based cervical cytology specimens. HPV Selfy HR was performed in combination with Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014). Relative sensitivity of HPV Selfy is 0.92 (96/119 on self-collected samples vs 104/119 on clinician-collected samples), whereas relative specificity is 1.03 (706/791 on self-collected samples vs 681/791 on clinician-collected samples).

Intra- and inter-laboratory reproducibility

Intra-laboratory reproducibility of the HPV Selfy HR assay was determined by analyzing a group of 523 self-collected vaginal swabs, which were analyzed twice within the same laboratory. The observed agreement is 96.7% (Cohen's kappa 0.882, excellent agreement). The inter-laboratory reproducibility of the HPV Selfy HR assay was determined by analyzing a group of 451 self-collected vaginal swabs (of which 25% positive), which were analyzed by two independent laboratories. The observed agreement is 90.7% (Cohen's kappa 0.739, substantial agreement).

Genotyping capability

To assess the genotyping ability of HPV Selfy HR, 59 randomly selected samples positive to HPV Selfy HR were tested with the CLART® HPV4 (Genomica, SAU; code #CS-0215-48) assay, which can provide genotyping information. CLART identified 88 different viral infections, while 81 infections were found by HPV Selfy HR. Overall, the agreement on HPV genotyping between the two tests was very good. In this subpopulation, the CLART test detected fewer HPV68 and HPV39 infections than HPV Selfy HR, while the HPV Selfy HR detected fewer HPV59 and HPV66 infections. We repeated HPV Selfy HR on the same samples to estimate the reproducibility of the genotyping and obtained substantial to perfect agreement with type-specific kappa in the range of 0.73 to 1.00.

References

Meijer et al. *Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older*. Int J Cancer (2009).

Arbyn et al. *VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples*. Journal of Clinical Virology 107 (2018).

Avian et al. *Clinical validation of full HR-HPV genotyping HPV Selfy assay according to the international guidelines for HPV test requirements for cervical cancer screening on clinician-collected and self-collected samples*. Journal of Translational Medicine (2022).

6. Explanations of symbols

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by date
	Temperature limit
CONTROL +	Positive Control (PC)
CONTROL –	Negative Control (NC)
	Consult instructions for use
	Manufacturer
	Contains sufficient for <n> tests
	Do not use if package is damaged and consult instructions for use

7. Contacts

Contact your local Ulisse Biomed representative for assistance.

Ulisse Biomed S.p.A.
Via Camillo Benso di Cavour 20
33100 - Udine (UD)
Italy

Customer Support & Technical Support: support@ulissebiomed.com

For more contact information visit www.ulissebiomed.com