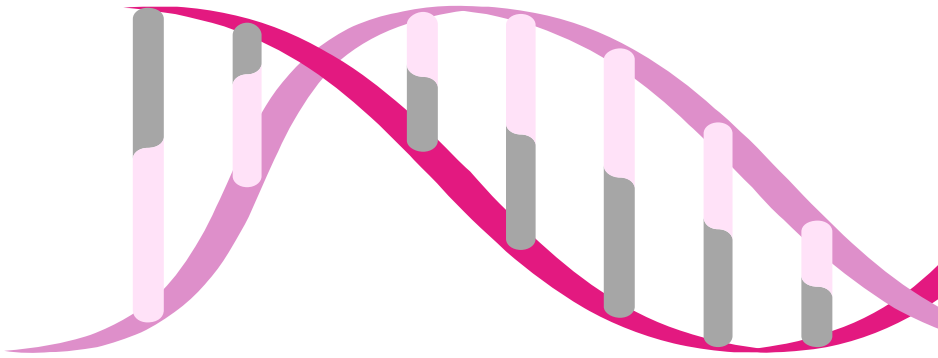
For Professional Use Only



ULISSE BioMed

We build using DNA: the molecule of life

Sagitta™

HPV Selfy 11 LR

INSTRUCTIONS FOR USE

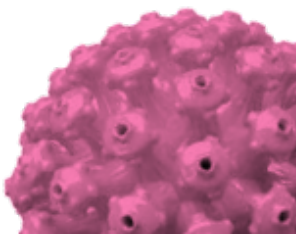
Multiplex real-time PCR assay for detection and genotyping of 11 low-risk HPV types from cervical swab, vaginal swab and liquid based cervical cytology specimens.

11 Low-risk HPV genotypes:
6, 11, 40, 42, 43, 44, 54, 61, 70, 81, 84

CE IVD

REF UBM0022

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



Version 2 | May 2023

DISCLAIMER

Ulisse Biomed S.p.A. reserves the right to modify its products and services at any time. This instruction manual is subject to change without notice. Although prepared to ensure accuracy, Ulisse Biomed S.p.A. assumes no liability for errors or for any damages resulting from the application or use of this information.

TRADEMARKS

Registered trademarks used in this document, even if not explicit, are not to be considered unprotected by law. This product and its use are covered by multiple Ulisse Biomed S.p.A. patents.

INDEX

1. Product description

Intended use

"HPV Selfy 11 LR" is an in vitro diagnostic (IVD) medical device intended for the qualitative multiplex detection and differentiation of nucleic acids from 11 Human Papillomaviruses (HPV), namely 11 low-risk HPV types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 81, 84), 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 11 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 11 LR 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 11 LR 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 11 LR is a multiplex real-time PCR assay that permits the simultaneous amplification, detection and differentiation of target nucleic acids of 11 HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70, 81, 84) as well as Internal Control (IC).

HPV Selfy 11 LR performs the amplification reaction starting from DNA extracted from each sample under test. HPV Selfy 11 LR 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 11 LR 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 11 LR assay consists of three PCR reactions:

- the first permitting the simultaneous amplification of target DNA of 6 low-risk HPV types (LR HPV 1 PCR Mix);
- the second permitting the simultaneous amplification of target DNA of 5 low-risk HPV types (LR HPV 2 - PCR Mix);
- the third 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 11 LR 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 11 LR 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 11 LR (Ulisse Biomed, S.p.A.; code #UBM0022-050) are sufficient for 50 tests, including controls.

HPV Selfy 11 LR (REF UBM0022-050)			
Contents	Volume	Description	Color
β-Globin Mix	1 X 0.35 mL	Buffered solution containing synthetic DNA for the specific amplification of β-Globin.	Pink
Reaction Mix (DNA)	2 X 1.50 mL	Buffered solution containing amplification and detection agents.	White
$MgCl_2$ 25mM solution	2 X 0.10 mL	Magnesium chloride 25mM solution.	Green
Negative Control	1 X 0.10 mL	Molecular-biological grade water.	White
LR HPV Mix 1	1 X 0.35 mL	Buffered solution containing synthetic DNA for the specific amplification of 6 low-risk HPV types.	Yellow
LR HPV Mix 2	1 X 0.35 mL	Buffered solution containing synthetic DNA for the specific amplification of 5 low-risk HPV types.	Yellow
Positive Control (HPV 42+53)	1 X 0.10 mL	Buffered solution containing synthetic DNA segments of HPV 42, HPV 53 and β-Globin (5,000 copies/µl).	Red
Positive Control (HPV 40+54)	1 X 0.10 mL	Buffered solution containing synthetic DNA segments of HPV 40, HPV 54 and β-Globin (5,000 copies/µl).	Red

Materials required but not provided

A. Materials required for every compatible system:

The following materials are required to use HPV Selfy 11 LR 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.
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.), or AriaDx Real-time PCR System (Agilent Technologies, Inc.)
- Ice.
- Disposable nitrile powder-free gloves, or similar material, and adequate personal protective equipment.

B. 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).

C. 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).

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 11 LR. 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 QuantStudio™ 5, Agilent AriaDx

The procedure to use HPV Selfy 11 LR 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 11 LR.

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 11 LR:

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

B. Preparation of amplification PCR mixes

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 three 1.5 mL polypropylene capped tubes which will contain the LR HPV PCR mix 1, the LR HPV PCR mix 2 and the ß-globin PCR Mix respectively; identify the tube with an indelible marker.

Prepare the LR HPV PCR Mix 1, the LR HPV PCR Mix 2 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.

LR HPV PCR Mix 1		
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
LR HPV Mix 1	5.40 µL	5.94 x (n + 2) µL
Total volume	18.00 µL	-
LR HPV PCR Mix 2		
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
LR HPV Mix 2	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, mix slowly by inversion at least 10 times, avoiding the formation of bubbles.

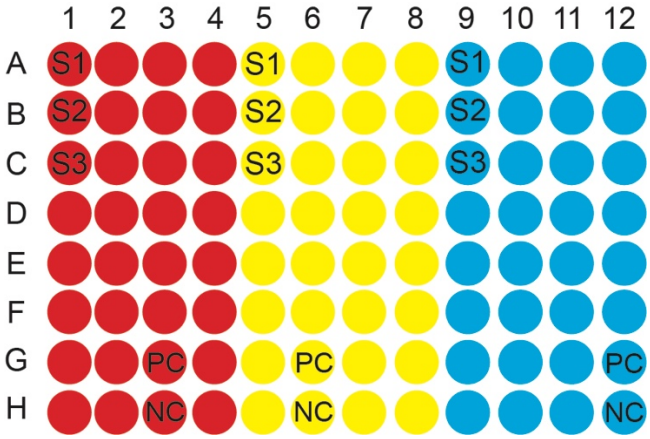
C. PCR plate assembly

Load 18 µL of LR HPV PCR Mix 1, 18 µL of LR HPV PCR Mix 2 and 18 µL of ß-globin PCR Mix for each sample in three separate wells.

Load three times 2 µL of each biological sample (S1, S2, S3, etc), of mix-specific Positive Control (PC) and of Negative Control (NC): once in the LR HPV PCR Mix 1-loaded well, once in the LR HPV PCR Mix 2-loaded well and once in the ß-globin PCR Mix-loaded well, as indicated in the figure below.

Use Positive Control (HPV40+54) in the LR HPV PCR Mix 1.

Use Positive Control (HPV42+53) in the LR HPV PCR Mix 2 and the ß-globin PCR Mix.



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
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 any LR HPV PCR Mix	Ct ß-globin PCR Mix	Tm any LR 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 low-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 low-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 low-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 targeted HPV type, can be further analyzed to determine specifically which HPV type(s) is present.

The HPV Selfy 11 LR assay allows to discriminate 11 HPV types, i.e.: HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 81, 84, by means of analysis of melting temperature (Tm) of the amplified DNA analyte. In the HPV Selfy 11 LR assay, each mix enables identification and differentiation of the targeted HPV types since each genotype is characterized by a specific Tm interval:

- LR HPV PCR Mix 1 detects and differentiates the following HPV types: HPV 40, 43, 44, 54, 61, 70;
- LR HPV PCR Mix 2 detects and differentiates the following HPV types: HPV 6, 11, 42, 81 and 84.

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 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

LR HPV PCR Mix 1					
Instrument					
HPV type	QuantStudio™ 5		**HPV type**	AriaDx	
	Melting Temperature °C			Melting Temperature °C	
	from	to		from	to
HPV61	72.50	74.00	HPV61	73.00	74.80
HPV70	74.05	75.50	HPV70	75.00	76.60
HPV40	77.00	79.00	HPV40	77.00	79.60
HPV43	79.50	81.00	HPV43	79.80	81.00
HPV54	81.05	82.50	HPV54	81.40	84.00
HPV44	85.50	87.00	HPV44	86.00	88.00
LR HPV PCR Mix 2					
Instrument					
HPV type	QuantStudio™ 5		**HPV type**	AriaDx	
	Melting Temperature °C			Melting Temperature °C	
	from	to		from	to
HPV42	76.00	77.20	HPV42	76.20	77.60
HPV6	79.00	80.00	HPV6	79.40	80.20
HPV11	81.10	82.00	HPV11	81.40	82.60
HPV84	84.50	86.00	HPV84	85.00	86.60
HPV81	88.00	89.00	HPV81	87.00	89.00
ß-globin PCR Mix					
Instrument					
Target	QuantStudio™ 5		**Target**	AriaDx	
	Melting Temperature °C			Melting Temperature °C	
	from	to		from	to
ß-globin	75.50	76.80	ß-globin	76.40	77.80

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 each of the three PCR Mixes. 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 for each PCR Mix. The analysis is considered valid when all the following conditions are met:

- Positive Control (HPV40+54) is characterized by amplification curves in the LR HPV PCR Mix 1 and the ß-globin PCR Mix.
- Positive Control (HPV42+53) is characterized by amplification curves in the LR HPV PCR Mix 2.
- Negative Control is characterized by no amplification curves neither in any LR HPV PCR Mix, nor in the ß-globin PCR Mix.

For a correct genotyping analysis, it is necessary to detect two melting peaks of the Positive Control (HPV40+54) (tested with LR HPV PCR Mix 1); one melting peak of the Positive Control (HPV42+53) (tested with LR HPV PCR Mix 2); and one melting peak of the Positive Control (HPV40+54) (tested with ß-globin PCR Mix), within the melting temperature ranges indicated below:

PCR Mix	Target	Melting Temperature Range °C			
		QuantStudio™ 5		AriaDx	
		from	to	from	to
LR HPV PCR Mix 1	HPV40	77.00	79.00	77.00	79.60
	HPV54	81.05	82.50	81.50	84.00
LR HPV PCR Mix 2	HPV42	76.00	77.20	76.20	77.60
ß-Globin PCR Mix	ß-Globin	75.50	76.80	76.40	77.80

If an amplification signal exceeding the threshold value for any LR 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 or Tm are observed in the Positive Control, the plate is invalidated, and it has to be repeated. In this case contact the supplier of the product.

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 any LR 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.

4. Limits

HPV Selfy 11 LR detects DNA of 11 low-risk HPV types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 81, 84). This test does not detect DNA of other HPV types. HPV Selfy 11 LR provides a qualitative result.

HPV Selfy 11 LR 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 11 LR.

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 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 11 LR 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 11 LR 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 11 LR 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 AriaDx Real-time PCR System (Agilent Technologies, Inc.) 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 11 LR was determined by spiking full genome HPV plasmids at known concentration. The LoD of HPV Selfy 11 LR is 500 copies/reaction for all HPV types, except for HPV40, 43, 51, 54, 61 (1,000 copies/reaction); and HPV44, 70 (3,000 copies/reaction).

Analytical specificity (cross-reactivity)

The potential cross-reactivity of the HPV Selfy 11 LR assay was evaluated through testing a panel of 17 bacteria, viruses and fungi, 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
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 11 LR 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 11 LR 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 11 LR 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 LR HPV PCR mix was evaluated on a library of 58 remnant clinical self-collected vaginal specimens, in combination with Ulisse Faster DNA direct kit (Ulisse BioMed DNA; ref. UBM0014). Reference test was CLART® HPV4 (Genomica, SAU; code #CS-0215-48) assay; in case of discordance, samples were sequenced. Of 27 positive samples, 26 were positive to LR HPV PCR mix; on 32 negative samples, 31 were negative for LR HPV PCR mix, with a total agreement of 96.6% (Cohen's kappa: 0.932, almost perfect agreement); relative diagnostic sensitivity and specificity are 96% and 97%, respectively.

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