Papilloplex® HR HPV Genotyping Kit

Catalogue # MPAHPV001

FOR *IN VITRO* DIAGNOSTIC USE

Detection and differentiation of 14 high-risk Human papillomavirus (HR HPV) types from patient specimens in a single assay

The 14 HR HPV targeted are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68

Store at -20°C

Protect from light

Instructions for Use – English

Version 5.0

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Glossary

IVD	*In-vitro* diagnostic
PCR	Polymer Chain Reaction
HPV	Human papillomavirus
HR	High Risk
MPA	Multiplex Probe Amplification
PC	Positive Control
NC	Negative Control
UNG	Uracil-N-Glycosylase
LOD	Limit of Detection

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Contents

1. Kit contents

Materials supplied within the kit:

Tube cap colour	Reagent	Description
Green	Buffer Mix	Mg^{2+}, dNTPs
Blue	Enzyme Mix	Taq Polymerase, UNG enzyme, dUTP
Amber	Working Mix	Primers and probes
Yellow	Negative control	TE Buffer
Red	Positive control	Control DNA

Additional equipment & reagents required (not provided in the kit):

- Reagents and equipment for specimen collection, filtration, and DNA extraction
- Water, distilled (molecular biology grade)
- DNase, RNase and human DNA-free pipette tips with aerosol barriers
- DNase, RNase and human DNA-free tubes for preparing Reaction Mix
- Pipettes (adjustable)
- Tube racks
- Vortex mixer
- Microcentrifuge
- Cool racks or ice
- Thermo Fisher Scientific, Applied Biosystems 7500 Real-Time PCR System
- Applied Biosystems 7500 Real-Time PCR System Sequence Detection Software v1.4
- PCR tubes, plates and accessories compatible with the use of the Applied Biosystems 7500 Real-Time PCR System

2. Shipment and storage

- Papilloplex® HR HPV Genotyping Kit is shipped frozen at -20° C using gel packs.
- Upon receipt of the kit, components must be stored in freezer at -20°C or below.
- The contents must be protected from light to prevent photobleaching and stored in the manufacturer's packaging.
- After opening, the Papilloplex® HR HPV Genotyping Kit is stable up to the expiration date indicated on the packaging provided that the components have been stored correctly according to the recommendations.

3. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted infections and high-risk (HR) types of HPV cause the majority of cases of cervical cancer. Accurate molecular detection of HR HPV infection is of great use for cervical cancer screening, monitoring treatment and epidemiological studies.

GeneFirst has developed the Multiplex Probe Amplification (MPA) technology enabling real-time PCR detection of multiple targets in a single closed-tube reaction. The Papilloplex® HR HPV Genotyping Kit can detect and differentiate all 14 HR HPV types in addition to a cellular control target in a single reaction.

3.1. Intended use

The Papilloplex® HR HPV Genotyping Kit is an in-vitro diagnostic (IVD) test for the detection and differentiation of 14 high-risk Human Papillomavirus (HR HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 from liquid-based cytology and first void urine specimens in a single assay.

3.2. Target environment

The Papilloplex® HR HPV Genotyping Kit may be used in testing clinical/pathology laboratories for applications such as primary screening, triage or test-of-cure. It may also be used for epidemiological studies in research settings.

3.3. Principle

The Papilloplex® HR HPV Genotyping Kit is based on the MPA technology patented by GeneFirst Limited. The MPA technology allows differentiation of up to six different targets per fluorescence channel, using a combination of PCR primers and probes (dual labelled fluorescent probe and partially complementary oligo hybrid) for each specific HR HPV target. Each probe has a unique melting profile (different melting temperature) that allows specific detection of the target present in the sample (Figure 1).

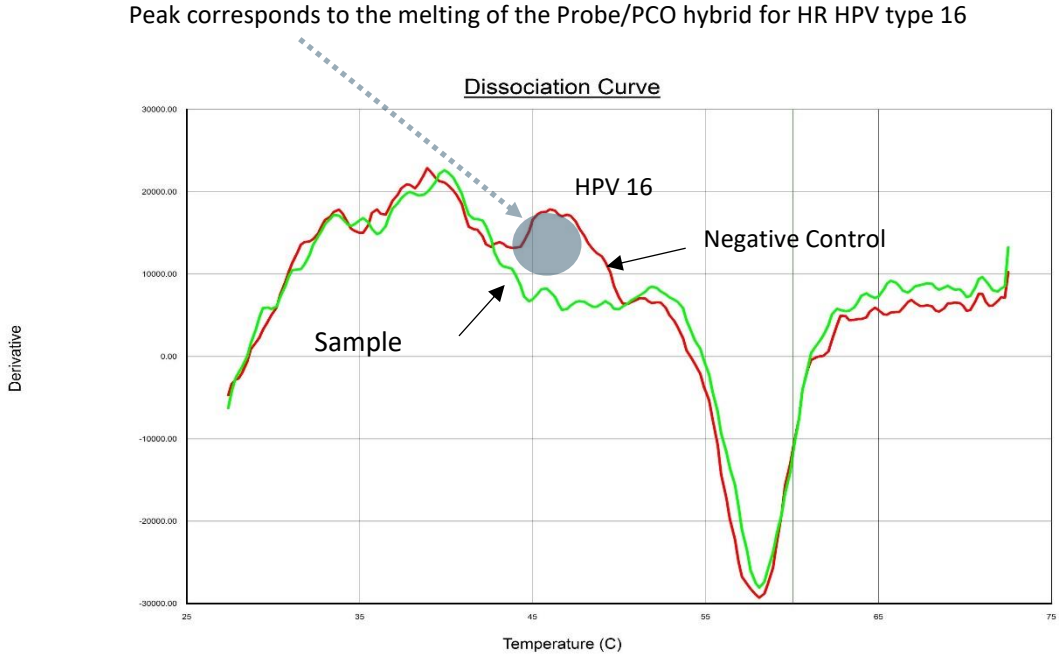


Figure 1. Melting curves/profiles in FAM channel. Y axis = derivative fluorescence and X axis = temperature. Red line denotes NC - (melting profile of reference) and green line denotes test sample/specimen. The difference (shown by grey circle) in the melting curve allows identification of the HR HPV genotype present in the sample tested (HR HPV type 16 in the figure).

Real-time PCR is performed in the standard manner thereby amplifying any HR HPV DNA targets present in the sample. If one or more targets are present, the corresponding probes are consumed during the amplification, producing a reduction in the signal visible in the final melt curve analysis. Comparing melting profiles of the probes reveals which specific probe is consumed, thus indicating which HR HPV target is present in a sample.

3.4. Controls provided in the kit

The tube with a **red** cap contains Positive control to be used in each PCR run. The control contains HR HPV genotypes 16, 58, 45 and human DNA as a linearized plasmid. They are detected in the FAM, JOE (HEX), ROX and Cy5 channels respectively.

The tube with a **yellow** cap contains Negative Control to be used in each PCR run. The results using the NC are used for establishing the reference melting curve profile for HR HPV genotyping.

4. Contraindications, warnings and precautions

4.1. Contraindications

There are no known contraindications identified.

4.2. Interfering substances

The performance of the kit may be adversely affected by known PCR inhibitors co-extracted from patients' samples (such as blood, acetic acid, iodine, excessive mucous or pharmaceutical preparations such as lubricant gels, spermicide creams etc). The use of samples containing such substances should be avoided.

4.3. Warning and precautions

- This kit is designed to be used for *in vitro* diagnostic use and should be used by trained personnel with good laboratory practice and good competency in real-time PCR.
- Upon arrival, please check the kit for signs of damage. If damaged, please contact GeneFirst customer service or your local distributor. Do not use damaged kit components as they may not yield the expected performance.
- The kit is not intended to be re-used.
- Do not use the product beyond its expiry date.
- Do not mix reagents from different batches.
- Negative and positive controls provided in the kit must be used as controls in experiments.
- Fluorescently labelled probes included in the Amber tube (Working Mix) are sensitive to photobleaching. Exposure to light should be avoided as much as possible.
- Lab coats and powder-free gloves must be worn at all times.
- Never touch the inside of the tube cap.
- Appropriate pipette tips with an aerosol barrier and free of DNase, RNase and human DNA must be used.
- Use appropriate measures to decontaminate working surfaces such as wipe/spray with 0.5% Sodium Hypochlorite solution or DNA AWAY™.
- Thaw all components thoroughly at room temperature before using the kit, mix and keep on ice.
- Avoid excessive vortexing.
- Disposal of unused reagents and waste must be done in accordance with country or local regulations.
- Safety Data Sheet (SDS) is available on request from either GeneFirst or your distributor.

5. Operating procedure

5.1. Specimen collection

The kit has been validated using samples prepared by ThinPrep®, following their recommended procedures, and first void urine samples collected using either a Colli-Pee™ device or standard urine collection cup. For details on specimen collection, please refer to relevant product details from your supplier.

5.2. DNA extraction

Two DNA extraction kits have been tested for use in conjunction with the Papilloplex® HR HPV Genotyping Kit using their standard manufacturer recommended protocol:

- NucliSENS® easyMAG® from bioMérieux
- QIAamp® DNA Mini Kit from Qiagen

For detailed protocols on using individual kits, please refer to product details from your relevant suppliers. First void urine samples need to be concentrated using Amicon® Ultra-4 50 K Centrifugal Filter using the manufacturer recommended protocol.

5.3. PCR Reaction Mix setup

PCR Reaction Mix is prepared according to the table below. All steps above are performed on ice with minimal exposure to light. Before use, the Reaction Mix should be fully thawed and mixed thoroughly by vortexing and briefly spun down.

Transfer 15 µl of the Reaction Mix into each of the wells of a PCR plate, followed by adding 5 µl of positive control (PC) or test sample or negative control (NC) into each well. At least one PC and two NC samples should be included per run.

Note: adding consistent and precise amounts of reagents and DNA or control is critically important for accurate genotyping results.

Tube Cap colour	Name	Volume per single reaction (µl)	Volume required for 96 reactions plus excess** (µl)
Green	Buffer Mix	5.00	500.00
Blue	Enzyme Mix	0.35	35.00
Amber	Working Mix	2.15	215.00
(Not supplied with the kit)	H_2O (molecular biology grade)	7.5	750.00
	Reaction Mix	**15**	1500.00
	And		
Red*	Positive Control	5	**n/a**
	Or		
Yellow*	Negative Control	5	
	Or		
N/A*	Test sample	5	

*5µl of sample is recommended and should be used in most experiments. However, 2-8 µl of sample or negative control may be used. The volume of water must then be adjusted to ensure that the total reaction volume is 20µl. Please add the same total amount of reagents plus DNA to all PCR vessels. The Positive Control should be used at 5µl and the volume of additional water adjusted to ensure that the total reaction volume is 20µl

**To compensate for any loss during pipetting it may be necessary to prepare an additional volume of reaction mix, a 5% excess is usually sufficient.

Note: Seal the PCR plate using PCR caps and centrifuge briefly. Every well should be sealed tightly to avoid evaporation. **Starlab** 96-Well PCR Plate (cat. E1403-0200) and 8-Strip PCR Caps (cat. I1400-0900) are recommended for good results. MicroAmp Fast Optical 96-Well Reaction Plate (0.1ml) from ThermoFisher is not suitable.

The Enzyme Mix contains Uracil-N-Glycosylase (UNG) and UNG Enzyme to prevent any carry-over contamination.

6.3.2 Dispense 15 µl of each reaction mix to each well of a PCR plate according to the layout shown below. Empty wells are greyed out.

6.3.3 Add 5 µl of either test sample, PC or NC to each well according to the PCR plate layout shown below.

5.4. Real-Time PCR instrument settings

The assay has been optimized for the Thermo Fisher Scientific, Applied Biosystems® 7500 Real-Time PCR System operating 7500 Fast System SDS Software Version 1.4. The sections of protocol describing run settings and data analysis parameters are specific for this system.

- Place the plate in the instrument.
- In 7500 Fast System SDS Software Version 1.4, the PCR run is performed under the Standard 7500 Run Mode with cycling conditions as described in the table below.
- During the run setup, in the Data Collection window, select "Stage 3 Step 2 (60.0 @ 0:33)".
- PCR volume is set to 20 µl and "none" is selected for passive reference.
- Select detection of fluorescence signal in the FAM, JOE, ROX and Cy5 channels for all wells in use.

Stage	Cycles	Temperature (°C)	Duration	Data collection
1 - Amplification stage	1	50	2 min	
		95	3 min	
2 - Amplification stage	9	95	6 sec	
		66	45 sec	
3 - Amplification stage	42	95	3 sec	
		60	33 sec	End-point point fluorescence collection
		63	15 sec	
4 - Dissociation stage	1	95	15 sec	
		25	1 min	Real-time point fluorescence collection from 25 °C to 75 °C
		75	15 sec	
		60	15 sec	

6. Data analysis

The data is processed using the software: Applied Biosystems® 7500 Fast System SDS Software Version 1.4. Analysis is carried out in accordance with the software's instruction manual.

6.1. Setting the baseline, threshold and *Ct* calling

Select "manual baseline" and set the baseline to 3-10 cycles (or other range as long as it excludes the background found in the early cycles of amplification, but does not overlap with the area in which the amplification signal begins to rise above background).

The cycle number at which a signal is detected above background fluorescence is termed the cycle threshold (Ct). Select "manual Ct" and set the threshold for Ct determination as close as possible to the base of the exponential phase. Otherwise, the threshold for Ct determination can be set up as follows:

- **Cy5 = 20,000**
- **FAM = 40,000**
- **JOE = 10,000**
- **ROX = 40,000**

Amplification in the Cy5 channel indicates the presence of human DNA that serves as an internal reaction control for each sample. Amplification in the FAM, JOE or/and ROX channels indicates the presence of the HR HPV DNA types targeted by the assay.

The test is considered as valid only if:

- The Negative Control(s) do not show amplification in any channel (Cy5, FAM, JOE and ROX); Ct undetermined or ≥ 39.0
- The Positive Control(s) show amplification in all channels (Cy5, FAM, JOE and ROX)
- Amplification of human DNA in the Cy5 channel has Ct value < 38.0
- Samples are considered positive for amplification if they give a Ct < 36.0 in the FAM, JOE or ROX channels. Above this value, the sample will be reported as negative/no HR HPV type detected/viral load below assay detection limit.

6.2. Genotyping via melting curve analysis

The term melting profile refers to the melting curves (per channel) generated during the dissociation stage of the reaction (from 25°C to 75°C). The melting profile obtained per channel is a combination of the melting data generated from the probes of all HR HPV types included in this given channel. Each HR HPV type has a unique melting profile represented by a unique melting temperature and shape. The change in this characteristic melting profile in the sample compared to the NC reference melting profile shows the sample to be positive for the respective HR HPV type(s).

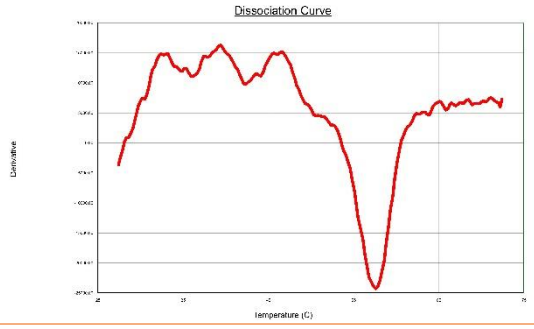
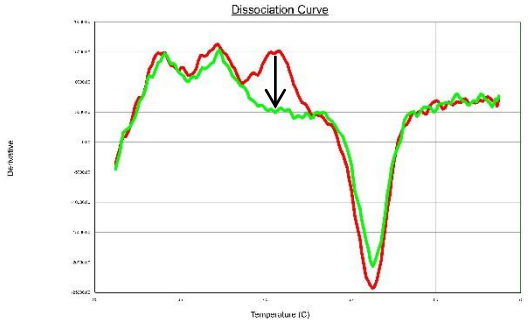
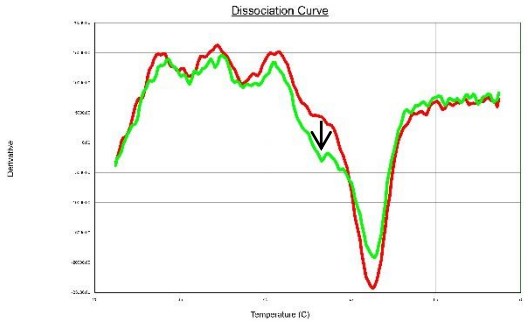
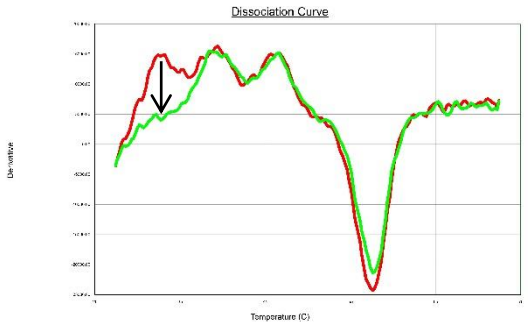
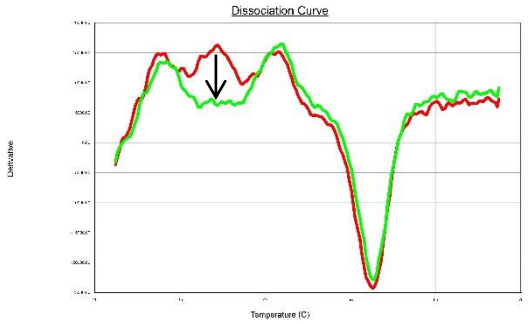
Follow the below steps in the SDS Software v1.4 to compare the melting curves of the sample vs NC:

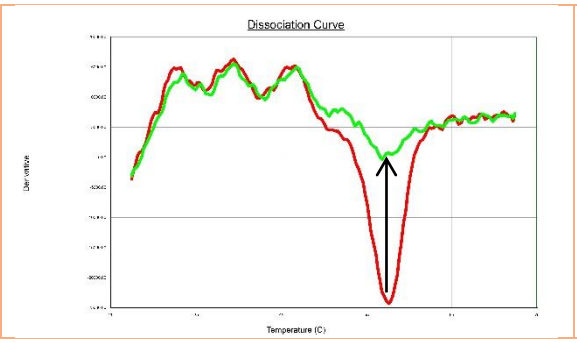
- Click on the 'Results' tab and select all the wells by clicking on the top left hand corner
- Click on 'Dissociation' tab
- Change 'Data Type' to 'Derivative'
- Right click on the graphs and click on Graph Settings.
- Untick 'Auto Scale' of X-axis and update the values to 25 °C (minimum) and 75 °C (maximum).
- Select one or two NC wells and compare with the melting profiles of individual samples in each channel.

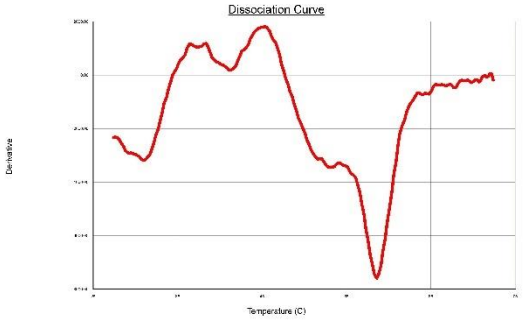
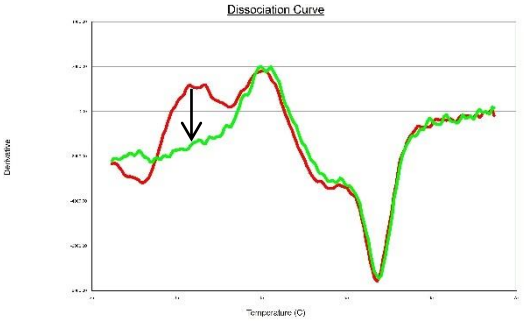
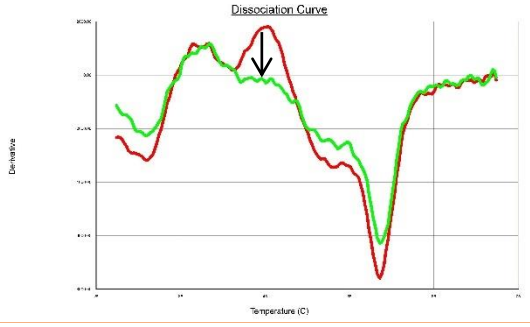
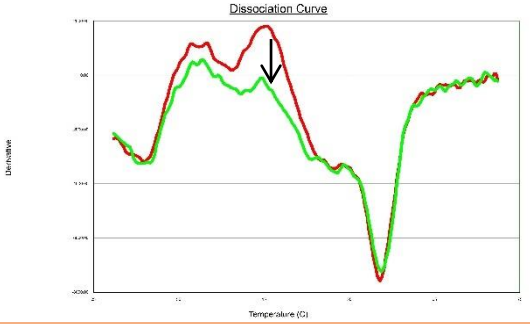
Each HR HPV type shows a characteristic melting profile in one channel which differs to the melting curve profile observed for the NC in the given channel. The differences in the melting curve profiles indicate the HR HPV type(s) present in the samples, as shown in the figures below.

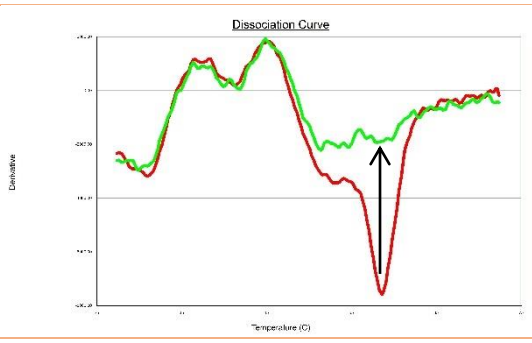
Specimen genotyping results are interpreted as shown below.

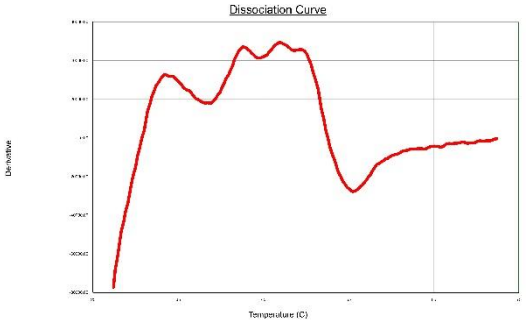
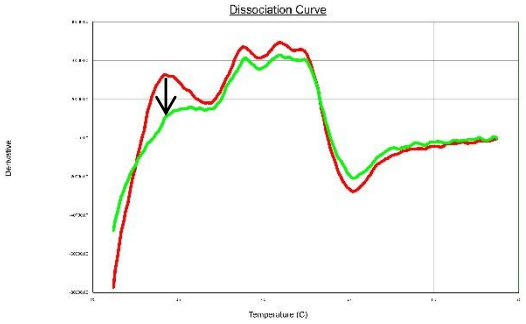
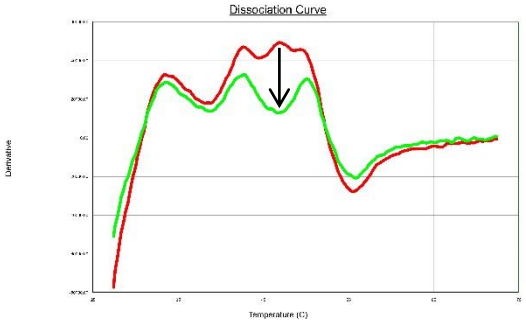
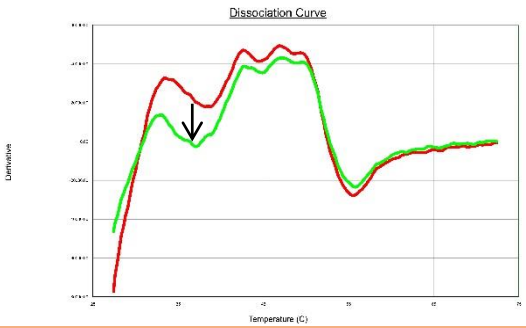
- Y axis denotes derivative fluorescence
- X axis shows temperature
- The **red line** is NC and the **green line** is a valid test result
- Exemplary melting curve profiles of NC that are suitable for analysis are shown below for each fluorescent channel
- Differences in melting curves that identify (genotype) HR HPV types are shown by arrows.

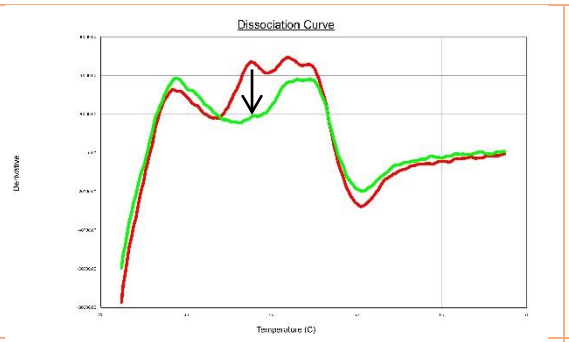
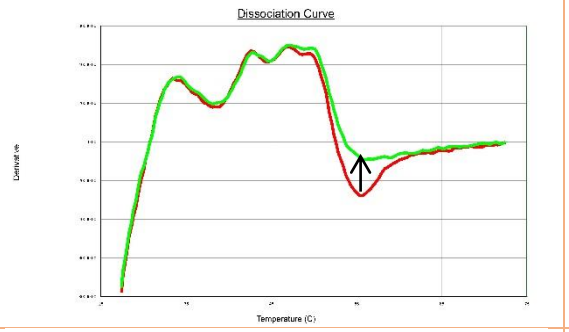
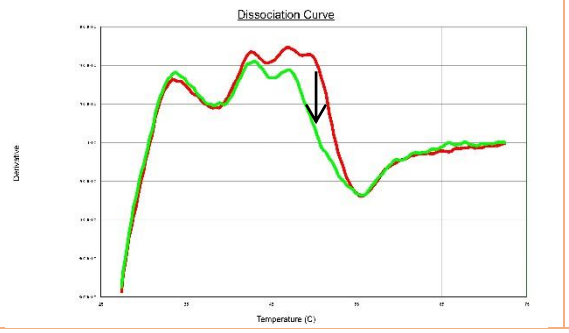
Melting curves for HR HPV types detected in the FAM channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NC melting profile		N/A
HPV 16		‘plus’ peak profile Tm: 46.3 ± 1.0 °C
HPV 18		‘plus’ peak profile Tm: 52.1 ± 1.0 °C
HPV 59		‘plus’ peak profile Tm: 32.8 ± 1.0 °C
HPV 31		‘plus’ peak profile Tm: 39.5 ± 1.0 °C

HPV 52		'minus' peak profile Tm: 57.6 ± 1.0 °C

Melting curves for HR HPV types detected in the JOE channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NC melting profile		N/A
HPV 39		'plus' peak profile Tm: 37.6 ± 1.0 °C
HPV 68a		'plus' peak profile Tm: 45.1 ± 1.0°C
HPV 68b		'plus' peak profile Tm: 46 ± 1.0°C

HPV 58		'minus' peak profile Tm: 58.7 ± 1.0°C

Melting curves for HR HPV types detected in the ROX channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NC melting profile		N/A
HPV 45		'plus' peak profile Tm: 33.5 ± 1.0°C
HPV 66		'plus' peak profile Tm: 46.8 ± 1.0°C
HPV 51		'plus' peak profile Tm: 36.2 ± 1.0°C

HPV 35		‘plus’ peak profile Tm: 42.8 ± 1.0°C
HPV 33		‘minus’ peak profile Tm: 55.8 ± 1.0°C
HPV 56		‘plus’ peak profile Tm: 50.3 ± 1.0°C

The table below gives information on sample results and suggested outcome in different scenarios for the Papilloplex® HR HPV Genotyping Kit.

Amplification in Cy5	Amplification in FAM, JOE or ROX	Changes in melting profile	Sample result and suggested actions
Yes	Yes	Yes	HR HPV Positive
Yes	No	No	HR HPV Negative
Yes	Yes	No	HR HPV Positive; must be re-tested for genotyping results
Yes	No	Yes	HR HPV Negative
No	Yes	No	Sample Invalid; must be re-tested
No	No	No	Sample Invalid; must be re-tested
No	No	Yes	Sample Invalid; must be re-tested

7. Analytical performance

7.1. Limit of detection

The limit of detection (LOD) of the Papilloplex® HR HPV Genotyping Kit was determined using quantified plasmid DNA for all 14 HR HPV genotypes. The claimed limit of detection per MPA reaction for HR HPV 16 and 18 is 100 copies. HR HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are detected at 1000 copies per MPA reaction.

7.2. Analytical specificity

Cross-reactivity with other HPV types was assessed using quantified plasmid DNA at 30,000 copies per MPA reaction for HPV 6, 10, 11, 26, 30, 32, 34, 40, 53, 54, 57, 61, 67, 69, 70, 73, 82, 85, 90 and 97. The results showed that the Papilloplex® HR HPV Genotyping Kit is specific to the targeted HR HPVs only.

Cross-reactivity was assessed with regards to non-HPV microorganisms reasonably expected to be present at the site of clinical sample collection. Quantified genomic DNA samples at 30,000 copies per MPA reaction of *Lactobacillus acidophilus, Candida albicans, Mycoplasma hominis, Trichomonas vaginalis C-1, Garnerella vaginalis, Staphylococcus aureus, Chlamydia trachomatis Serovar E, Human herpes virus 2, Escherichia coli,* and *Neisseria gonorrhoeae* were tested. The results showed that the Papilloplex® HR HPV Genotyping Kit is specific to the targeted HR HPVs only.

7.3. Repeatability

A repeatability study using the Papilloplex® HR HPV Genotyping Kit was completed with three consecutive runs performed by the same operator on the same instrument and using the same batch of the kit. Correct HPV types were detected in all samples (100% correct genotyping results). Total % coefficient of variation of Ct values of the low, medium and high copy number samples was below 2.5%.

7.4. Reproducibility

Verification of reproducibility was performed over 12 consecutive days, three times per day, by three operators on two instruments using three batches of the Papilloplex® HR HPV Genotyping Kit. The study consisted of 36 runs in total and each HR HPV type was assessed at three concentrations, low (1-10 time the LOD; 1,000 copies/reaction), medium (10-100 times the LOD; 10,000 copies/reaction) and high (>100 times the LOD; 100,000 copies/reaction). For all targeted HR HPVs a 99% positive hit rate was observed at low and medium concentrations (98% positive for high concentration) and the results are summarised in the table below. No significant variability between operators, runs and batches was recorded at any concentration tested.

Target	Copies per reaction	N	HPV Positive n (%)	HPV Negative n (%)
HPV 16	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)
HPV 18	100,000	108	107 (99%)	1 (1%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	108 (100%)	0 (0%)
HPV 31	100,000	108	108 (100%)	0 (0%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	107 (99%)	1 (1%)
HPV 33	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)
HPV 35	100,000	108	107 (99%)	1 (1%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)
HPV 39	100,000	108	108 (100%)	0 (0%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	107 (99%)	1 (1%)
HPV 45	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	108 (100%)	0 (0%)
HPV 51	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)
HPV 52	100,000	108	107 (99%)	1 (1%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)
HPV 56	100,000	108	108 (100%)	0 (0%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	107 (99%)	1 (1%)
HPV 58	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	108 (100%)	0 (0%)
HPV 59	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	108 (100%)	0 (0%)
HPV 66	100,000	108	107 (99%)	1 (1%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	108 (100%)	0 (0%)
HPV 68a	100,000	108	106 (98%)	2 (2%)
	10,000	108	107 (99%)	1 (1%)
	1,000	108	107 (99%)	1 (1%)
HPV 68b	100,000	108	108 (100%)	0 (0%)
	10,000	108	108 (100%)	0 (0%)
	1,000	108	107 (99%)	1 (1%)

7.5. Accuracy – Clinical specimens

7.5.1. Liquid based cytology specimens

100 clinical samples were tested by two operators and the results were compared to those from two commercial assays, the Abbott RealTi*m*e High Risk HPV and the Roche LINEAR ARRAY® HPV Genotyping Test. In detection of HR HPV, the Papilloplex® HR HPV Genotyping Kit shows 95% agreement with Abbott and Roche assays. Diagnostic sensitivity, specificity and proportional agreements between the Papilloplex® HR HPV Genotyping Kit and the two other assays are shown in the tables below.

		Roche LINEAR ARRAY® HPV		
		Positive	Negative	**Total**
Papilloplex® HR HPV Genotyping Kit	Positive	63	5	**68**
	Negative	1	31	**32**
	Total	**64**	**36**	**100**
Proportional Agreement		94%		
Kappa		0.87	95% CI (0.76, 0.97)	
Sensitivity		98%	95% CI (91,100)	
Specificity		86%	95% CI (70,95)	

		Abbott RealTi*m*e High Risk HPV		
		Positive	Negative	**Total**
Papilloplex® HR HPV Genotyping Kit	Positive	62	6	**68**
	Negative	2	30	**32**
	Total	**64**	**36**	**100**
Proportional Agreement		94%		
Kappa		0.8221	95% CI (0.70, 0.93)	
Sensitivity		96%	95% CI (89, 99)	
Specificity		83%	95% CI (67, 93)	

7.5.2. First void urine specimens

100 clinical samples from first void urine specimens were tested by two operators and the results were compared to those from the DiaMex Optiplex HPV Genotyping Kit and TS qPCR HPV Genotyping Test (A.M.L. bvba). In detection of HR HPV, the Papilloplex® HR HPV Genotyping Kit shows 92% proportional agreement with the Optiplex HPV Genotyping Kit and TS qPCR HPV Genotyping Test. Diagnostic sensitivity, specificity and proportional agreements between the Papilloplex® HR HPV Genotyping Kit and the two other assays are shown in the tables below.

		DiaMex Optiplex HPV Genotyping Kit		
		Positive	Negative	**Total**
Papilloplex® HR HPV Genotyping Kit	Positive	75	2	**77**
	Negative	16	7	**23**
	Total	**91**	**9**	**100**
Proportional Agreement		82%		
Kappa		0.3539	95% CI (0.13, 0.57)	
Sensitivity		82%	95% CI (73,90)	
Specificity		78%	95% CI (40,97)	

		TS qPCR HPV Genotyping Test		
		Positive	Negative	**Total**
Papilloplex® HR HPV Genotyping Kit	Positive	66	11	**77**
	Negative	7	16	**23**
	Total	**73**	**27**	**100**
Proportional Agreement		82%		
Kappa		0.521	95% CI (0.32, 0.72)	
Sensitivity		90%	95% CI (81, 96)	
Specificity		59%	95% CI (39, 78)	

8. Troubleshooting

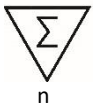
Should you encounter problems, please consult the table below:

Observation	Probable Cause	Solution
Absence of amplification of the human control	Inadequate DNA extraction method used	Section 7.2 outlines the methods with which the kit has been tested.
	Presence of PCR Inhibitors	The performance of the kit may be adversely affected by known PCR inhibitors co-extracted from patient's samples (such as blood, excessive mucous or pharmaceutical preparations such as lubricant jellies, spermicide creams etc.) We suggest repeating the test or obtaining a new patient sample/DNA extraction.
	Not enough human DNA in sample	Repeat the same sample with a higher test sample volume or obtain a new patient sample.
	If the results show amplification (*Ct* < 25) for FAM/JOE/ROX and significant changes in melting profile suggesting an HPV genotype, it is possible that competition from an excess of viral DNA may have repressed human DNA amplification	Results Valid
	No human DNA extracted	Repeat processing of the same sample or obtain a new patient sample.
	Instrument faulty	Check the instrument calibration records and confirm it is working.
	Kit stored at wrong temperature or under wrong conditions	Check the storage temperature and whether the contents were exposed to prolonged direct sunlight. Also ensure the reagents are kept on ice during use and avoid excessive vortexing.
	Incorrect PCR cycling parameters	Verify that PCR cycling parameters correspond to those recommended in the IFU.
Melting profile does not resemble the reference NC profile	Two adjacent changes in the melting curve can make it appear different from the reference profile	Proceed with caution. Two HR HPV genotypes may be present in the sample.
Shift in melting profile temperature/amplitude between sample and NC	Unequal volume of liquid in tubes due to e.g. evaporation of liquid from one tube or pipetting errors	Proceed with caution. If the melting curves are not possible to visually align, repeat the samples and NC.

9. Specifications of Papilloplex® HR HPV Genotyping Kit

Technology	Real-Time PCR
Target Sequence	L1 region
Specificity Range	95% to 100% for all 14 genotypes covered
Sensitivity (LOD)	for HPV 16 -100 DNA copies per reaction for HPV 18 – 100 DNA copies per reaction
Extraction/Inhibition Controls Included	Internal human DNA control for confirming absence of PCR inhibitors, sample adequacy and quality of DNA Extraction
Sample Material	No Sample material supplied with the Kit, however Genefirst recommends the following DNA Extraction methods: • NucliSENS® easyMAG® from bioMérieux • QIAamp® DNA Mini Kit from Qiagen
Kit Storage	-20 °C
Validated Real-Time PCR Devices	Thermo Fisher Scientific Applied Biosystems 7500 Real-Time PCR System
Quality Control	Positive and negative controls of amplification and genotyping
Certification	**CE** IVD for *in vit*ro Diagnostics Use

10. Symbols

	Consult instructions for use
	Catalogue number
	Date of manufacture
	Manufacturer
	Use-by-date
	*In vitro* diagnostic medical device
	Contains sufficient for n tests
	Upper limit of temperature -20 °C
	Keep away from sunlight
	Batch code
	Do not re-use
	Do not use if package damaged

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11. Customer contact information

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