Papilloplex® HR-HPV DNA Kit

Catalogue # (REF): MPAHPV006

FOR *IN VITRO* DIAGNOSTIC USE

Store at -20°C

Protect from light

Instructions for Use – English

Version 5.0

genefirst

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1. Trademarks

Papilloplex® is a registered mark of GeneFirst Ltd registered in the UK and Ireland.
ThinPrep® is a registered mark property of Hologic Inc.
DNA AWAY™ is a trademark property of Molecular Bio-Products Inc.

2. Copyright

3. Kit contents

Materials supplied with the kit:

Tube colour	Tube cap colour	Reagent	Description
Transparent	Green	Enzyme Mix	DNA Polymerase, buffer and dNTPs
Amber	Amber	Working Mix	Primers and probes
Transparent	Orange	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
- Real-Time PCR System: Clinical Performance evaluation have been performed on Bio-Rad CFX96 Real-Time PCR detection Systems. Analytical performance evaluation has been performed on both Bio-Rad CFX96 Real-Time PCR detection Systems and Shanghai Hongshi MedicalSlan-96P Real-Time PCR System.
- Real-Time PCR System Sequence Detection Software: CFX Manager software-IVD v1.6 and SLAN-96P 8.2.2
- PCR tubes, plates and accessories compatible with the use of the Real-Time PCR System
- Disposable powder-free gloves and lab-coat.

4. Shipment and storage

- Papilloplex® HR-HPV DNA Kit is shipped with frozen 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 DNA Kit is stable up to the expiration date indicated on the packaging provided that the components have been stored correctly according to the recommendations.

5. 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. Based on their frequency in cervical cancer, 14 types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) has been defined as carcinogens and, therefore, named as high-risk HPV (hrHPV) types.

The Papilloplex® HR-HPV DNA Kit is a real-time PCR test for genotyping of all the 14 hrHPV types in a single reaction tube.

5.1. Intended use

The Papilloplex® HR-HPV DNA Kit is a single reaction qualitative *in vitro* multiplex real-time polymerase chain reaction-based test for the screening of 14 individual high-risk (HR) Human Papillomavirus (HPV) types in human cervical samples (E6/E7 region). For professional use only.

5.2. Target environment

The Kit is for professional use only and may be used in pathology laboratories or in epidemiological studies in research settings.

5.3. Principle

The Papilloplex® HR-HPV DNA 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).

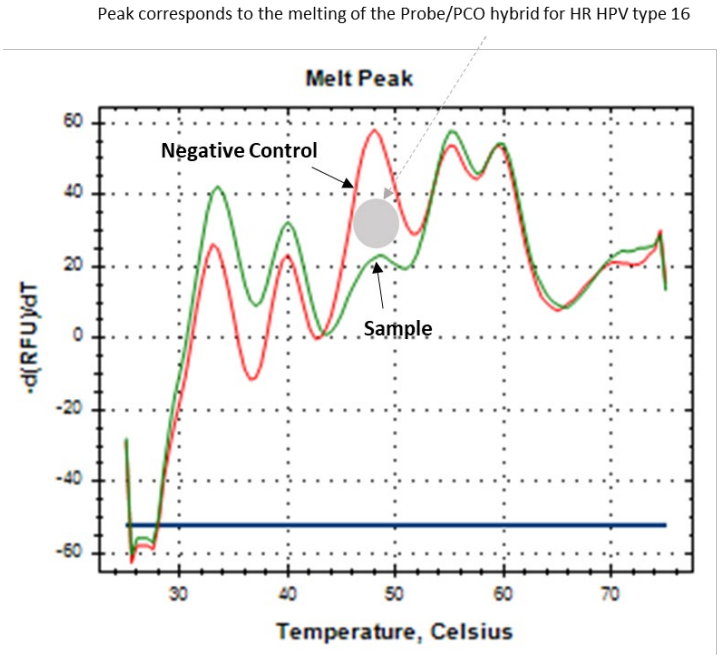


Figure 1. Melting curves/profiles in FAM channel. Y axis = derivative fluorescence and X axis = temperature. Red line denotes Negative Control - (melting profile of no template control, NTC) and green line denotes test sample/specimen. The difference (shown by grey circle) in the melting curve allows identification of the HR-HPV genotype present in the sample tested (HR-HPV type 16 in the figure).

5.4. Positive Control provided in the kit

The tube with an orange cap contains Positive control to be used in each PCR run. The control contains HR HPV genotypes 16, 45, 18 and GAPDH synthetic DNA. They are detected in the FAM, JOE (HEX), ROX and Cy5 channels, respectively.

6. Contraindications, warnings and precautions

6.1. Contraindications

There are no known contraindications identified.

6.2. 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.

6.3. Warning and precautions

- This kit is designed to be used for *in vitro* diagnostic 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.
- Partially used kit can be re-used if stored at -20°C following initial use. Thawing and freezing more than 4 times is not recommended. Prepared reaction mixes are for single use only and are not intended to be re-used.
- Do not use the product beyond its expiry date.
- Do not mix reagents from different batches.
- The positive control provided in the kit must be used as control in every experiment.
- 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 always be worn.
- 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 and mix.
- 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.

7. Operating procedure

7.1. Specimen collection

The kit has been validated on LBC samples conserved in PreservCyt transport medium (ThinPrep®). For details on specimen collection, please refer to relevant product details from your supplier.

7.2. DNA extraction

Two DNA extraction kits have been tested for use in conjunction with the Papilloplex®HR-HPV E6/E7 DNA Kit using their standard manufacturer recommended protocol:

- Quick DNA/RNA Viral Kit from Zymo Research

- Viral-PrepAdem-Kit from Ademtech

For detailed protocols on using individual kits, please refer to product details from your relevant suppliers.

7.3. PCR Reaction Mix setup

PCR Reaction Mix is prepared according to the table below. All steps above are performed at room temperature 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 designated well of a PCR plate, followed by adding 5 µl of positive control (PC) or test sample into each well. At least one PC 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	Enzyme Mix	4.00	**400.00**
Amber	Working Mix	2.00	**200.00**
(Not supplied with the kit)	H_2O (molecular biology grade)	9.00	**900.00**
	Reaction Mix	**15**	**1500.00**
And			
Orange*	Positive Control	5	**n/a**
Or			
N/A*	Test sample	5	
Or			
N/A*	H_2O (molecular biology grade)	5	

*5µl of sample is recommended and should be used in most experiments. However, 2-9 µl of sample may be used. The volume of water must then be adjusted to ensure that the total reaction volume is 20 µl. 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 20 µl

**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. **4titude** 96-Well PCR Plate (cat. 4ti-0750/TA) and 8-Strip PCR Caps (cat. 4ti-0751) are recommended for good results.

7.4. Real-Time PCR instrument settings

The assay has been optimized for Bio-Rad CFX96 Real-Time PCR detection Systems and Shanghai Hongshi MedicalSlan-96P Real-Time PCR System. The sections of protocol describing run settings and data analysis parameters are specific for these systems.

7.4.1. Real-Time PCR instrument settings for Bio-Rad CFX96

- Place the plate in the instrument.
- PCR volume is set to 20 µl and “none” is selected for passive reference.
- Select **all channels** detection for all wells in use.
- The PCR run is performed using the Standard Run Mode with cycling conditions as described in the table below

Stage	Cycles	Temperature (°C)	Duration	Data collection
Hotstart	1	95	3 min	
Amplification	48	95	10 sec	
		60	30 sec	End-point point fluorescence collection
		69	15 sec	
Dissociation	1	95	15 sec	
		25	30 sec	Real-time point fluorescence collection from 25°C to 75°C
		75	5 sec	

7.4.1.1. Software for Data analysis

Perform data analysis using Bio-Rad CFX Manager (Version 3.1).

7.4.1.2. Sample analysis

Amplification in the FAM, HEX and ROX channels indicates the presence of the viral DNA targeted by the assay.

Amplification in Cy5 channel serves as an internal PCR reaction control for each sample. Every tested samples should give a Cy5 signal (Ct<35) from DNA of a human endogenous internal control (IC) gene. For NTC: No amplification (or Ct> 38) should be detected in FAM, HEX or ROX channel and no amplification or Ct>35 should be detected in CY5 channel.

7.4.1.3. Setting the baseline, threshold and Ct calling

The baseline should be set to a range that eliminates the background fluorescence found in the early cycles of amplification, but which does not overlap the area where amplifications signals rise above the background. Baseline may be set automatically if it gives a suitable value.

The cycle number at which a signal is detected above background fluorescence is termed the cycle threshold (Ct). Select the threshold for Ct determination as close as possible to the base of the exponential phase. As an indication, select a threshold that gives in all channels Ct values for PC between 25 and 33.

Obtain threshold cycle (Ct) values for each channel, the test is considered valid only if:

- NTC shows no amplification (or Ct >38) in FAM, HEX and ROX channels and NTC shows no amplification (or Ct >35) in Cy5 channel.
- Positive Control shows amplification in FAM, HEX, ROX and Cy5 channels (Ct<35).
- Amplification of Internal Control in clinical sample is <35 Ct in Cy5 channel.
- Samples are considered positive for the amplification if they give a Ct value ≤ 39.0.

7.4.1.4. Viral identification via melting profile 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 all viral types included in this channel. Each viral type has a unique melting profile consisting of a unique melting temperature and shape. A change in this characteristic melting profile, in comparison with the NTC reference melting profile, shows the sample to be positive for the respective viral type(s).

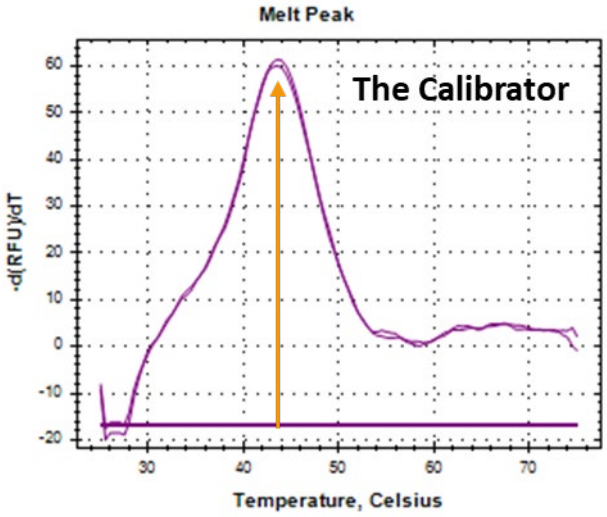
The characteristic melting profiles for each viral type are shown in the figures below:

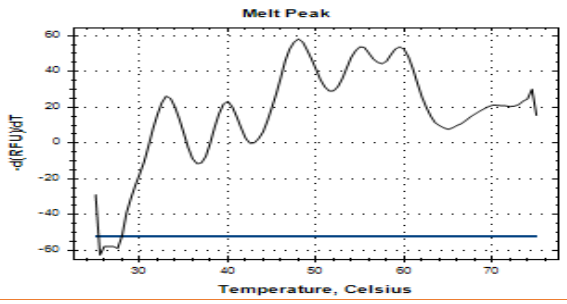
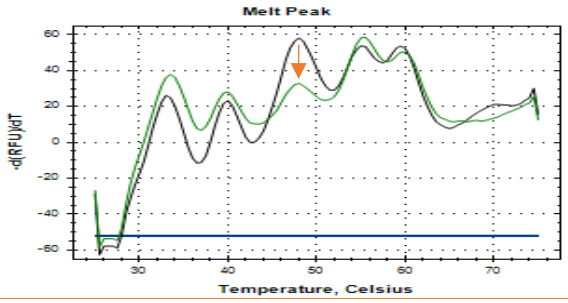
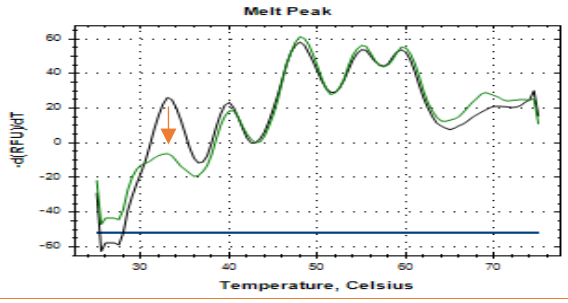
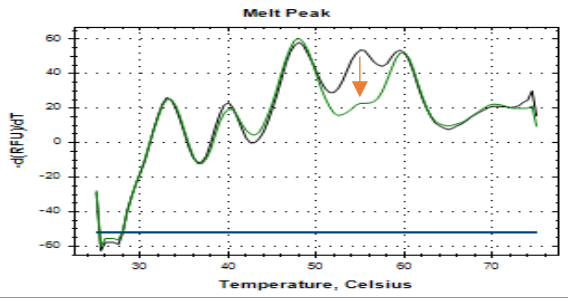
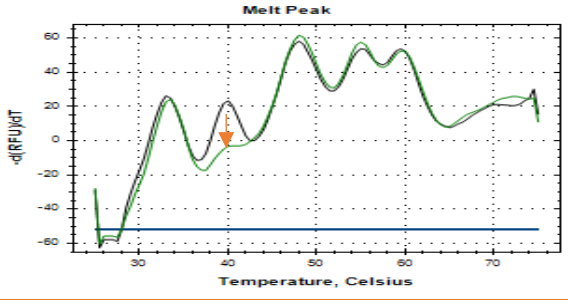
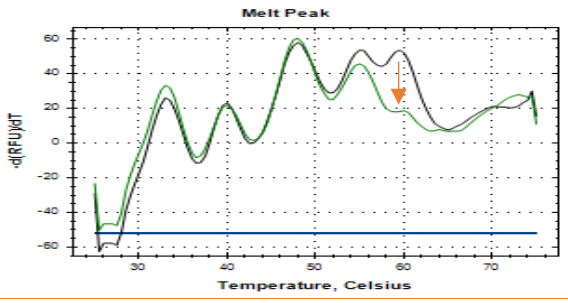
Specimen genotyping results are interpreted as shown below.

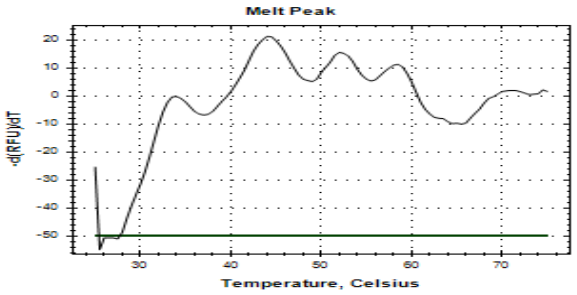
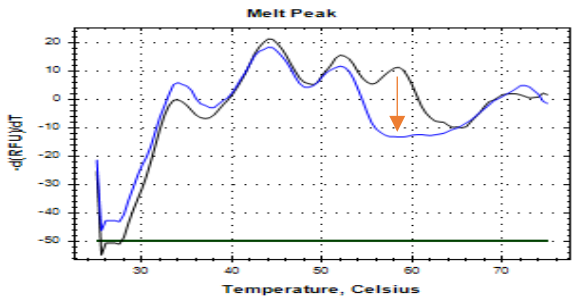
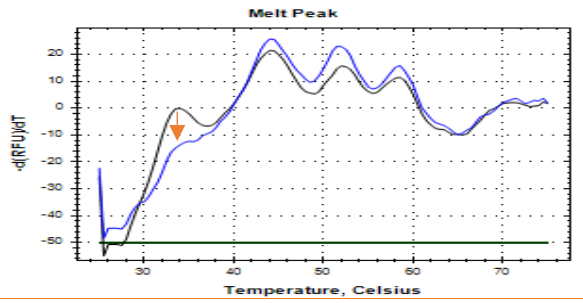
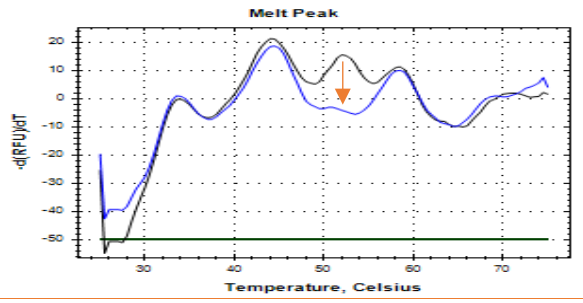
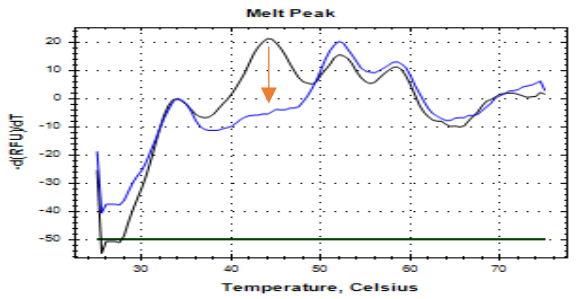
- Y axis denotes derivative fluorescence
- X axis shows temperature
- Melting curve profiles of NTC that are suitable for analysis are shown below for each fluorescent channel

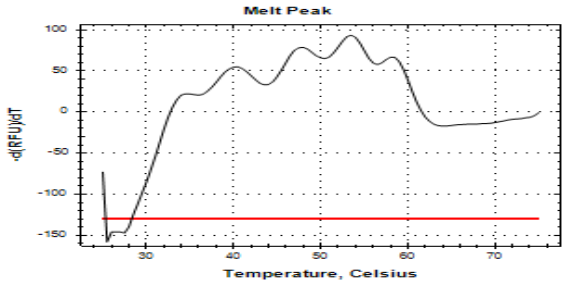
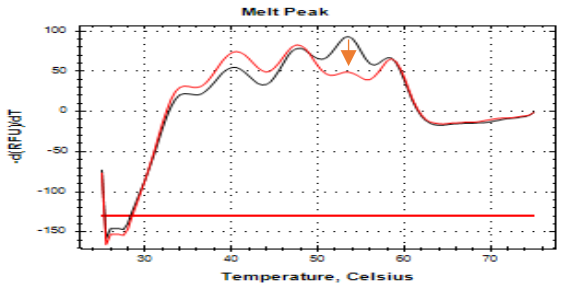
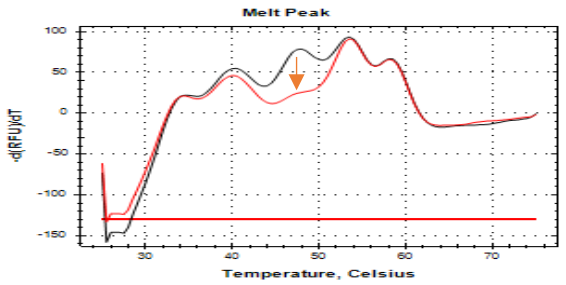
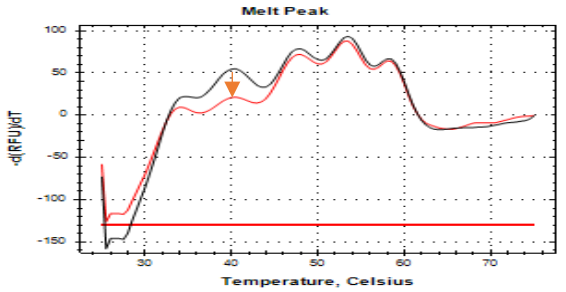
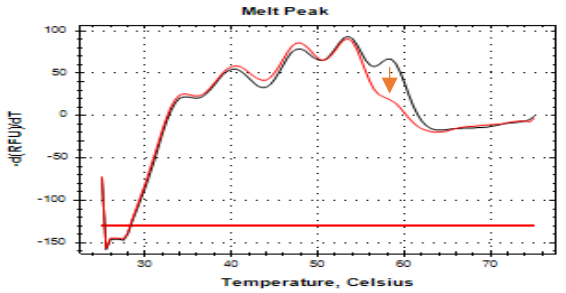
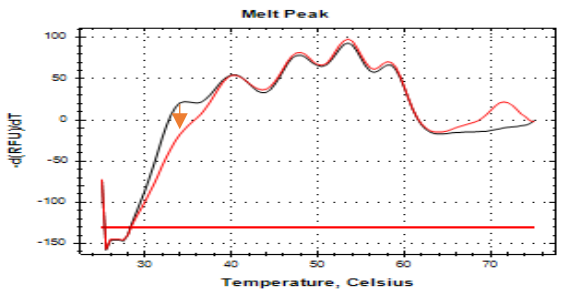
- Differences in melting curves that specifically identify viral types are indicated by arrows

First, check the melting curve profiles of the calibrator in Cy5 channel, the peak is usually detected at 43.0±0.5 °C (see Figure below). If the peak has shifted to a lower or higher temperature, a similar shift will be observed in all the melting curves in all channels and so should be taken into consideration during the analysis.



Melting curves for viral types detected in FAM channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 16 (HPV16)		48.0 ± 1.0 °C
Human Papillomavirus type 31 (HPV31)		34.0 ± 1.0 °C
Human Papillomavirus type 66 (HPV66)		55.0 ± 1.0 °C
Human Papillomavirus type 39 (HPV39)		40.0 ± 1.0 °C
Human Papillomavirus type 59 (HPV59)		59.0 ± 1.0 °C

Melting curves for viral types detected in HEX channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 56 (HPV56)		Tm: 58.0 ± 1.0 °C
Human Papillomavirus type 33 (HPV33)		Tm: 34.0 ± 1.0°C
Human Papillomavirus type 45 (HPV45)		Tm: 53.0 ± 1.0°C
Human Papillomavirus type 68 (HPV68)		Tm: 44.0 ± 1.0°C

Melting curves for viral types detected in ROX channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 52 (HPV52)		Tm: 54.0 ± 1.0°C
Human Papillomavirus type 18 (HPV18)		Tm: 48.0 ± 1.0°C
Human Papillomavirus type 35 (HPV35)		Tm: 40.0 ± 1.0°C
Human Papillomavirus type 51 (HPV51)		Tm: 59.0 ± 1.0°C
Human Papillomavirus type 58 (HPV58)		Tm: 34.0 ± 1.0°C

7.4.2. Real-Time PCR instrument settings for SLAN96P

- Place the plate in the instrument.
- PCR volume is set to 20 µl and “none” is selected for passive reference.
- Select **all channels** detection for all wells in use.
- The PCR run is performed using the **Melting Curve Mode** with cycling conditions as described in the table below

Stage	Cycles	Temperature (°C)	Duration	Data collection
Hotstart	1	95	3 min	
Amplification	48	95	10 sec	
		60	30 sec	End-point point fluorescence collection
		69	15 sec	
Dissociation	1	95	15 sec	
		27	30 sec	Real-time point fluorescence collection every 0.5°C (stepwise) from 27°C to 65°C

7.4.2.1. Software for data analysis

Perform data analysis using SLAN Real-time PCR System (Version 8.2.2).

7.4.2.2. Sample analysis

Amplification in the FAM, HEX and ROX channels indicates the presence of the viral DNA targeted by the assay.

Amplification in Cy5 channel serves as an internal PCR reaction control for each sample. Every tested samples should give a Cy5 signal (Ct<35) from DNA of a human endogenous internal control (IC) gene. For NTC: No amplification (or Ct> 38) should be detected in FAM, HEX or ROX channel and no amplification or Ct>35 should be detected in CY5 channel.

7.4.2.3. Setting the baseline, threshold and Ct calling

The baseline should be set to a range that eliminates the background fluorescence found in the early cycles of amplification, but which does not overlap the area where amplifications signals rise above the background. Baseline may be set automatically if it gives a suitable value.

The cycle number at which a signal is detected above background fluorescence is termed the cycle threshold (Ct). Select the threshold for Ct determination as close as possible to the base of the exponential phase. As an indication, select a threshold that gives in all channels Ct values for PC between 25 and 33.

Obtain threshold cycle (Ct) values for each channel, the test is considered valid only if:

- NTC shows no amplification (or Ct >38) in FAM, HEX and ROX channels and NTC shows no amplification (or Ct >35) in Cy5 channel.
- Positive Control shows amplification in FAM, HEX, ROX and Cy5 channels (Ct<35).
- Amplification of Internal Control in clinical sample is <35 Ct in Cy5 channel.
- Samples are considered positive for the amplification if they give a Ct value ≤ 39.0.

7.4.2.4. Viral identification via melting profile analysis

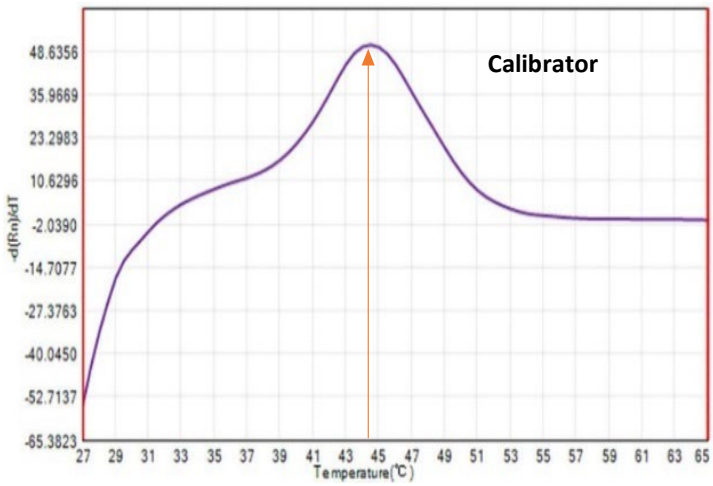
The term melting profile refers to the melting curves (per channel) generated during the dissociation stage of the reaction (from 25°C to 75°C). The melting profile obtained per channel is a combination of the melting data generated from all viral types included in this channel. Each viral type has a unique melting profile consisting of a unique melting temperature and shape. A change in this characteristic melting profile, in comparison with the NTC reference melting profile, shows the sample to be positive for the respective viral type(s).

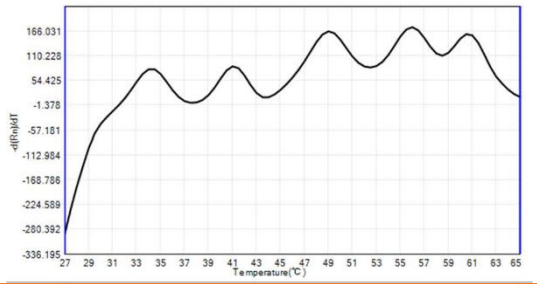
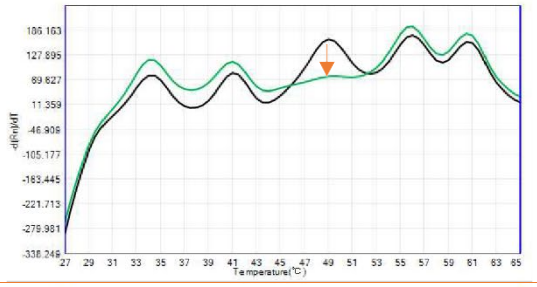
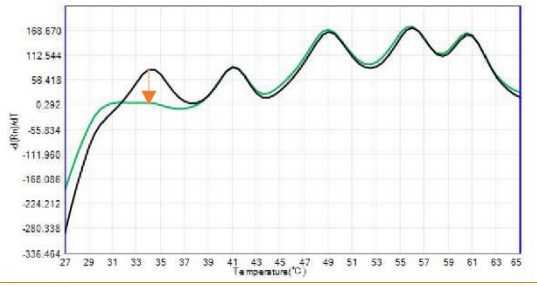
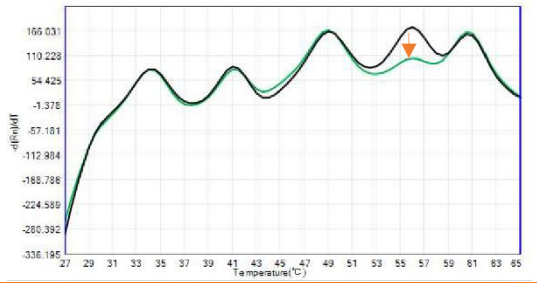
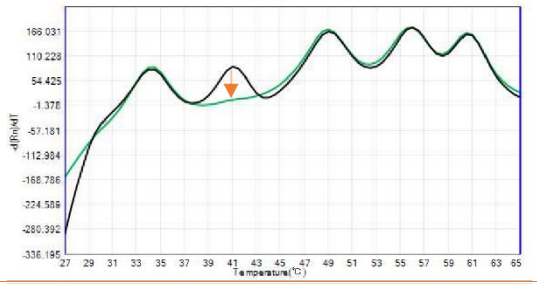
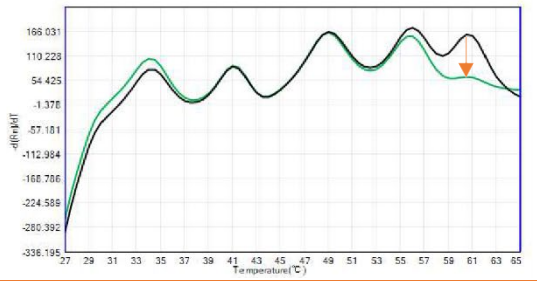
Each viral type shows a characteristic melting profile which differs to the melting curve observed for the NTC in the given channel. The difference between the melting curve profiles indicates the viral type present in the sample as shown in the figures below.

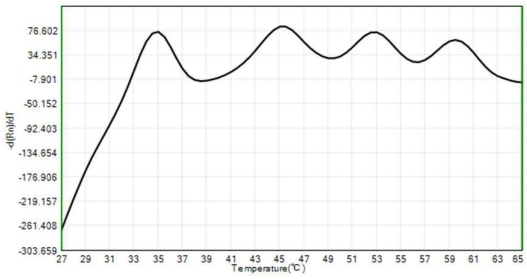
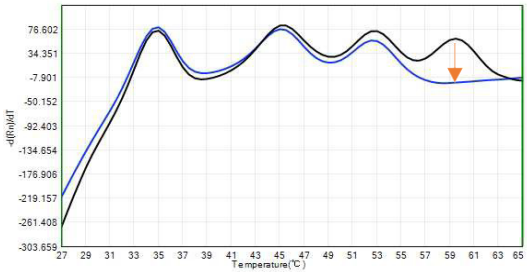
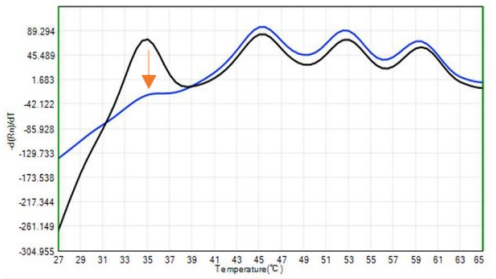
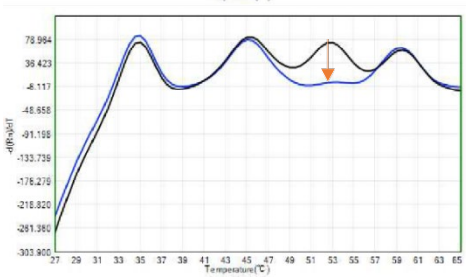
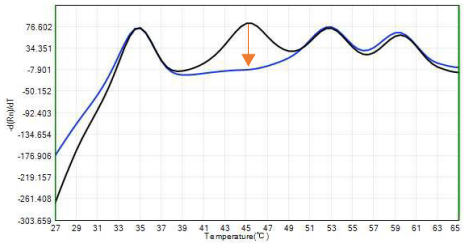
Specimen genotyping results are interpreted as shown below.

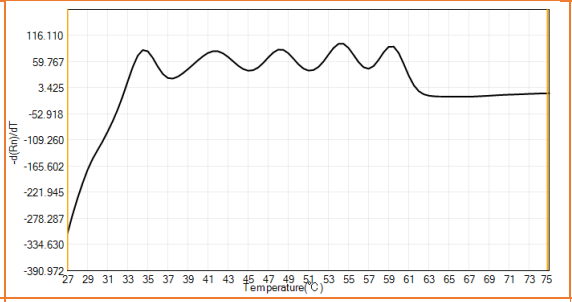
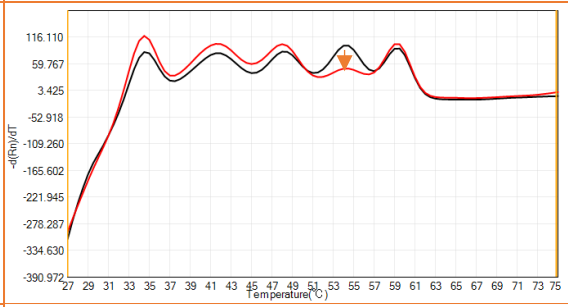
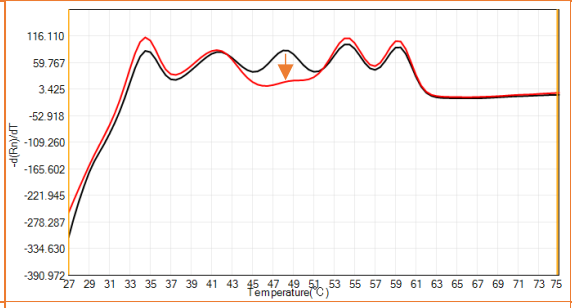
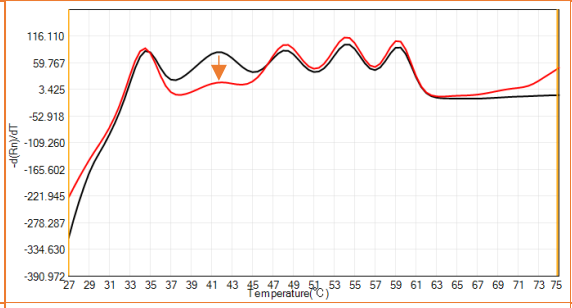
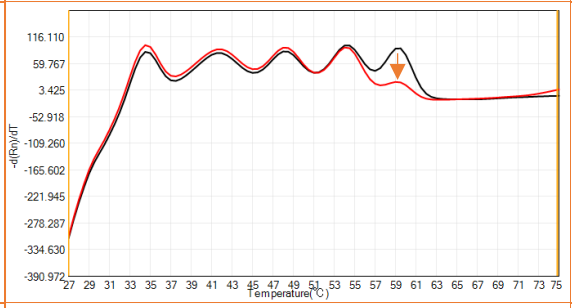
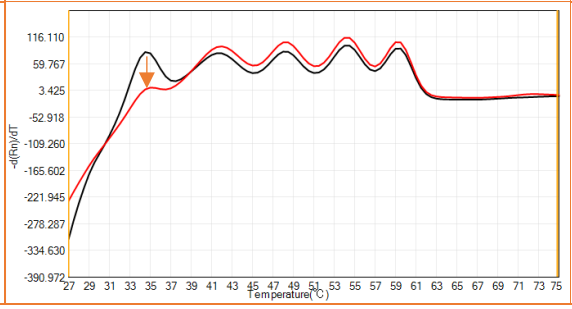
- Y axis denotes derivative fluorescence
- X axis shows temperature
- Melting curve profiles of NTC that are suitable for analysis are shown below for each fluorescent channel
- Differences in melting curves that specifically identify viral types are shown by arrows

First, check the melting curve profiles of the calibrator in Cy5 channel, the peak is usually detected at 44.0±0.5 °C. If the peak has shifted to a lower or higher temperature, a similar shift will be observed in all the melting curves in all channels and so should be taken into consideration during the analysis.



Melting curves for viral types detected in FAM channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 16 (HPV16)		48.0 ± 1.0 °C
Human Papillomavirus type 31 (HPV31)		35.0 ± 1.0 °C
Human Papillomavirus type 66 (HPV66)		55.0 ± 1.0 °C
Human Papillomavirus type 39 (HPV39)		40.0 ± 1.0 °C
Human Papillomavirus type 59 (HPV59)		60.0 ± 1.0 °C

Melting curves for viral types detected in HEX channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 56 (HPV56)		Tm: 59.0 ± 1.0 °C
Human Papillomavirus type 33 (HPV33)		Tm: 34.0 ± 1.0°C
Human Papillomavirus type 45 (HPV45)		Tm: 53.0 ± 1.0°C
Human Papillomavirus type 68 (HPV68)		Tm: 45.0 ± 1.0°C

Melting curves for viral types detected in ROX channel		
Genotyping result	**Representative melting curve profile**	**Features**
Standard/expected NTC melting curve profile		N/A
Human Papillomavirus type 52 (HPV52)		Tm: 54.0 ± 1.0°C
Human Papillomavirus type 18 (HPV18)		Tm: 48.0 ± 1.0°C
Human Papillomavirus type 35 (HPV35)		Tm: 42.0 ± 1.0°C
Human Papillomavirus type 51 (HPV51)		Tm: 59.0 ± 1.0°C
Human Papillomavirus type 58 (HPV58)		Tm: 34.0 ± 1.0°C

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

Amplification in Cy5	Amplification in FAM, HEX or ROX	Changes in melting profile	Sample result and suggested actions
Yes	Yes	Yes	HPV positive. Virus type detected.
Yes	No	No	HPV negative.
Yes	Yes	No	Potentially HPV positive. Virus type undetected; one of the targets in that channel may be present, re-testing or manual inspection of the amplification curve and melt curve is recommended
Yes	No	Yes	HPV negative.
No	Yes	Yes	HPV positive. Virus type detected.
No	Yes	No	Sample invalid; not enough nucleic acid, should be re-tested
No	No	No	Sample invalid; not enough nucleic acid, should be re-tested
No	No	Yes	Sample invalid; not enough nucleic acid, should be re-tested

8. Troubleshooting

Should you encounter problems please consult the table below:

Observation	Probable Cause	Solution
Absence of amplification in test samples	Presence of PCR inhibitors	The performance of the kit may be adversely affected by known PCR inhibitors co-extracted from patient samples (such as blood, excessive lubricant gels, spermicide creams, etc.) We suggest repeating the test or obtaining a new patient sample/nucleic acid extraction.
	Insufficient nucleic acid in test sample	Repeat processing of the same sample using a greater test volume or obtain a new patient sample.
Absence of amplification in positive control	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 above.
Melting profile that does not resemble the reference NTC melt profile	Two adjacent changes in melting curves can make it appear different from the reference profiles	Two or more viral types might be present in the sample.
	Unequal volume of liquid in tubes due to 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 NTC.

9. Performance

9.1. Stability

The purpose of stability testing is to provide evidence on how the quality of Papilloplex® HR-HPV DNA Kit varies under the different environmental factors such as temperature or freeze-thawing.

Papilloplex® HR-HPV DNA Kit could undergo 4 cycles of thaw-freeze without any loss in performance.

Papilloplex® HR-HPV DNA Kit could go through 8h at 37°C, 12h at room temperature and freeze again at -20°C without any loss in performance.

Papilloplex® HR-HPV DNA Kit could be set-up at room temperature up to 30°C without any loss in performance.

After setting-up the Papilloplex® HR-HPV DNA Kit , a delay in starting the PCR reaction (2 hours 40 min) does not affect the performance of the kit.

9.2. Limit of Detection (LOD)

The Limit of detection of Papilloplex® HR-HPV DNA Kit was determined on the Bio-Rad CFX96 real-time PCR instrument using quantified DNA plasmids containing E6/E7 partial sequences for each HR-HPV targeted by the assay.

Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
HPV16	200 000	22.7	0.4	2.1
	10 000	28.5	0.5	1.3
	500	32.3	0.6	1.7
	100	35.0	0.8	2.2
HPV18	200 000	21.7	0.3	1.4
	10 000	27.0	0.3	1.2
	500	30.9	0.3	1.0
	100	33.9	1.6	4.8
HPV31	200 000	23.3	0.5	2.2
	10 000	28.7	0.2	0.8
	500	32.7	0.5	1.5
	100	36.1	0.6	1.7
HPV33	200 000	23.7	0.3	1.4
	10 000	28.9	0.2	0.7
	500	32.9	0.4	1.3
	100	35.7	0.6	1.6
HPV35	200 000	20.4	0.3	1.6
	10 000	24.5	0.5	2.0
	500	29	0.4	1.4
	100	31.6	0.8	2.5
HPV39	200 000	23.1	0.3	1.4
	10 000	27.3	0.5	1.9
	500	32.3	0.4	1.3
	100	35.3	0.4	1.0
HPV45	200 000	24.2	0.2	1.0

Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
	10 000	28.5	0.4	1.5
	500	33.6	0.6	1.9
	100	36.2	0.7	2.0
HPV51	200 000	25.3	0.5	2.1
	10 000	30	0.8	2.6
	500	35.4	0.5	1.5
	100	38.9	0.8	2.0
HPV52	200 000	23.7	0.5	2.1
	10 000	29.4	0.3	1.2
	500	33.8	0.5	1.5
	100	37.1	0.7	1.8
HPV56	200 000	23.8	0.2	1.0
	10 000	28.6	0.4	1.5
	500	33.7	0.3	0.9
	100	36.5	0.7	1.8
HPV58	200 000	21.0	0.4	1.9
	10 000	25.8	0.4	1.7
	500	30.3	0.4	1.4
	100	32.5	0.6	1.9
HPV59	200 000	22.9	0.2	1.0
	10 000	27.4	0.8	3.1
	500	32.3	0.6	1.7
	100	35.3	0.7	1.9
HPV66	200 000	23.5	0.5	2.2
	10 000	28.1	0.6	2.0
	500	32.7	0.5	1.5
	100	35.2	0.6	1.7
HPV68	200 000	22.3	0.2	1.0
	10 000	26.7	0.8	3.1
	500	31.8	0.4	1.3
	100	34.4	0.5	1.6

9.3. Analytical Specificity (cross-reactivity with others HPV types)

The BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).search showed that the primers and the probes included in the Papilloplex® HR-HPV DNA Kit do not cross-react with all sequences known for low-risk HPV types. The only HPV type that might pose a risk of cross-reactivity, however very low, is HPV97. The HPV97 is an extremely rare high-risk type that has been reported only in women in Costa Rica (Chen *et al.,* 2007) and HIV positive men in Canada (Gorska-Flipot *et al.,* 2008).

9.4. Analytical Specificity (cross-reactivity with other microorganisms)

Potential cross-reactivity with non-HPV microorganisms reasonably expected to be present at the site of clinical sample collection was assessed, using quantified genomic DNA samples at 10 000 copies per 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*. No cross-reactivity were observed.

9.5. Precision

9.5.1. Precision Intra-Assay

The precision Intra-assay was performed by one operator on one instrument using one batch of Papilloplex® HR-HPV DNA Kit. The study consisted of 3 runs per instrument and 3 concentrations were assessed: Low (10 times LOD; 1000 copies/reaction), Medium (100 times the LOD; 10 000 copies/reaction) and high (>100 times the LOD; 100 000 copies per reaction). The results are summarised below:

Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
HPV16	100 000	24.2	0.5	2.0
	10 000	27.8	0.7	2.4
	1 000	31.2	0.5	1.5
HPV18	100 000	23.0	0.2	0.9
	10 000	26.6	0.7	2.6
	1 000	30.0	0.7	2.3
HPV31	100 000	24.0	0.4	1.7
	10 000	28.2	0.6	2.0
	1 000	32.0	0.4	1.4
HPV33	100 000	25.3	0.9	3.6
	10 000	28.3	0.4	1.3
	1 000	32.0	0.5	1.6
HPV35	100 000	21.6	0.4	1.8
	10 000	24.3	0.6	2.4
	1 000	28.1	0.3	1.2
HPV39	100 000	24.0	0.3	1.4
	10 000	27.6	0.6	2.2
	1 000	31.7	0.5	1.7
HPV45	100 000	24.8	0.5	2.1
	10 000	28.4	0.4	1.5
	1 000	32.6	0.3	0.9
HPV51	100 000	26.9	0.7	2.7
	10 000	30.3	0.4	1.2
	1 000	34.8	0.4	1.1
HPV52	100 000	25.6	0.7	2.6
	10 000	29	0.6	2.0
	1 000	32.9	0.3	1.0
HPV56	100 000	24.6	0.3	1.4
	10 000	28.6	0.5	1.7
	1 000	32.3	0.4	1.3
HPV58	100 000	22.4	0.4	1.6
	10 000	25.8	0.7	2.7
	1 000	29.4	0.4	1.5

Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
HPV59	100 000	23.7	0.7	2.8
	10 000	27.3	0.4	1.3
	1 000	31.5	0.5	1.6
HPV66	100 000	24.4	0.4	1.5
	10 000	27.9	0.6	2.2
	1 000	31.7	0.3	0.9
HPV68	100 000	23.4	0.2	0.9
	10 000	27.1	0.3	1.1
	1 000	30.6	0.4	1.2

9.5.2. Precision Inter-Assay

Verification of Reproducibility was performed over 8-days, three time per day by two operators on two instruments using three batches of Papilloplex® HR-HPV DNA Kit. The study consisted of 24 runs per platform (Bio-Rad CFX96 or Slan96P) and 3 concentrations were assessed: Low (10 times LOD; 1000 copies/reaction), Medium (100 times the LOD; 10 000 copies/reaction) and high (>100 times the LOD; 100 000 copies per reaction). No significant variability between operators, runs and batches was recorded at any concentration tested.

The results are summarised below:

Instruments	Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
Bio-Rad CFX96	HPV16	100 000	24.8	1.1	4.4
		10 000	28.6	1.4	4.9
		1 000	31.7	0.8	2.5
	HPV18	100 000	22.8	0.6	2.8
		10 000	26.5	1.0	3.8
		1 000	29.9	0.9	2.9
	HPV31	100 000	24.7	0.6	2.4
		10 000	28.5	0.9	3.3
		1 000	32.1	1.0	3.1
	HPV33	100 000	25.6	0.6	2.5
		10 000	29.1	0.8	2.7
		1 000	32.7	1.1	3.4
	HPV35	100 000	21.0	0.5	2.5
		10 000	24.2	0.7	2.8
		1 000	27.4	0.6	2.1
	HPV39	100 000	24.5	0.6	2.6
		10 000	27.9	0.7	2.5
		1 000	31.2	0.6	1.8
	HPV45	100 000	25.2	0.6	2.5
		10 000	28.4	0.8	2.8
		1 000	32.6	1.0	3.0

Instruments	Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
	HPV51	100 000	26.0	0.7	2.9
		10 000	29.7	0.8	2.6
		1 000	33.3	1.4	4.0
	HPV52	100 000	24.8	0.9	3.5
		10 000	29.3	1.5	5.1
		1 000	32.5	0.7	2.2
	HPV56	100 000	25.5	0.7	2.7
		10 000	29.6	0.9	3.1
		1 000	33.5	1.3	3.8
	HPV58	100 000	22.1	0.5	2.3
		10 000	25.3	0.7	2.8
		1 000	29.1	0.7	2.2
	HPV59	100 000	24.7	0.9	3.7
		10 000	28.0	1.1	4.0
		1 000	31.1	0.8	2.5
	HPV66	100 000	24.9	0.6	2.4
		10 000	28.0	0.8	2.8
		1 000	31.5	0.6	1.9
	HPV68	100 000	24.3	1.1	4.4
		10 000	27.6	1.1	3.9
		1 000	30.8	0.7	2.3
SLAN 96P	HPV16	100 000	21.8	0.9	4.2
		10 000	25.3	0.6	2.4
		1 000	29.1	1.0	3.5
	HPV18	100 000	17.7	0.6	3.5
		10 000	21.6	1.1	4.9
		1 000	24.6	0.5	2.2
	HPV31	100 000	22.4	0.9	3.9
		10 000	26.0	0.6	2.5
		1 000	29.9	0.8	2.5
	HPV33	100 000	23.1	0.8	3.6
		10 000	26.7	0.8	2.9
		1 000	30.5	0.8	2.7
	HPV35	100 000	16.2	0.3	1.8
		10 000	19.7	0.4	2.1
		1 000	22.9	0.5	2.2
	HPV39	100 000	21.6	0.8	3.7
		10 000	25.0	0.9	3.7
		1 000	28.5	1.3	4.6
	HPV45	100 000	21.8	0.8	3.7
		10 000	25.5	1.2	4.8
		1 000	29.7	2.1	7.2
	HPV51	100 000	20.0	0.7	3.6

Instruments	Target	Concentration Copies per reactions	Ct (Mean)	Standard Deviation	CV %
		10 000	23.6	0.8	3.5
		1 000	27.4	1.0	3.8
	HPV52	100 000	19.7	0.8	4.0
		10 000	23.7	0.5	2.3
		1 000	27.6	1.0	3.5
	HPV56	100 000	21.7	0.7	3.2
		10 000	26.1	1.2	4.6
		1 000	29.5	0.8	2.7
	HPV58	100 000	17.1	0.5	2.7
		10 000	20.5	0.5	2.4
		1 000	24.5	0.6	2.5
	HPV59	100 000	21.5	0.6	2.8
		10 000	25.3	1.5	6.1
		1 000	28.7	2.2	7.8
	HPV66	100 000	24.7	0.9	3.5
		10 000	28.4	1.2	4.3
		1 000	31.8	1.5	4.6
	HPV68	100 000	20.03	0.5	2.4
		10 000	23.7	0.6	2.5
		1 000	27.2	1.1	3.9

9.6. Clinical Performance

9.6.1. Clinical specimens

225 samples [LBC in PreserCyt medium (ThinPrep®)] were obtained from the Scottish HPV reference laboratory. Nucleic acids were extracted using either Quick DNA/RNA Viral Kit from Zymo Research or Viral-PrepAdem-Kit from Ademtech. Samples were previously tested with RealTime High-Risk HPV assay (Abbott Molecular). 200 samples were tested positive and 25 were tested negative with High-Risk HPV assay (Abbott Molecular).

A summary of the distribution of the samples according to their Cytology and Histology diagnoses is presented below:

Cytology/Histology	Not available	Cytology Neg so no Histology	Negative for dysplasia	Koilocytosis	CIN1	CIN2	CIN3
Negative	0	85	0	0	0	0	0
Bordeline Squamous	29	0	6	1	3	1	0
Low grade dysk	3	0	10	1	2	0	4
High Grade moderate dysk	0	0	6	1	3	6	5
High Grade severe dysk	0	0	1	0	2	15	41

9.6.2. Performance of Papilloplex® HR-HPV DNA Kit compared to comparator test

		Abbott				
	Status	Negative	Positive	Positive Percent agreement	Negative percent agreement	Overall percent agreement (CI)
GeneFirst	Negative	25	11	95.0 (91.3-97.5)	86.2 (68.3-96.1)	94.9 (91.0-97.4)
	Positive	4	210			

9.6.3. Performance of Papilloplex® HR-HPV DNA Kit compared to Histology results (CIN2+)

	Sensitivity % (CI)	Specificity % (CI)	Predictive Positive Value (PPV) % (CI)	Negative Predictive Value (NPV) % (CI)
Abbott	100 % (95.0-100)	20.7% (13.8-29.0)	42,9% (40.7-45.1)	100% (95-100)
GeneFirst	100 % (95.0-100)	29.8% (21.8-38.7)	45.9% (43-48.75)	100% (95-100)

Comparison	outcome	parameter	relative accuracy
GeneFirst vs Abbott	CIN2+	relative sensitivity	1
GeneFirst vs Abbott	CIN3+	relative sensitivity	0.983
GeneFirst vs Abbott	≤CIN1	relative specificity	1.041

10. Product Specification

Technology	Real-Time PCR
Target Sequence	E6/E7 region
Clinical Sensitivity	100%
Sensitivity (LOD)	100 copies per reactions
Kit Storage	-20 °C
Sample Material	No Sample material supplied with the Kit, however Genefirst recommends the following DNA Extraction methods: - Quick DNA/RNA Viral Kit from Zymo Research - Viral-PrepAdem-Kit from Ademtech
Validated Real-Time PCR Devices	Biorad-CFX96 and SLAN96P
Quality Control	Positive and internal cellular controls of PCR amplification and sample integrity
Certification	**CE** IVD for *in vitro* Diagnostics Use

11. Symbols

	Consult instructions for use		Upper limit of temperature -20°C
	Catalogue number		Keep away from sunlight
	Date of manufacture		Batch code
	Manufacturer		In vitro diagnostic medical device
	Use-by-date		Do not use if package damaged
	Authorised representative in the European Community		

Please Note: Information in these instructions for use is subject to change without notice and does not represent commitment on the part of GeneFirst. No part of these instructions for use may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying and recording for any purpose without the written permission of GeneFirst.

12. Customer contact information

For all sales order processing, training and technical support enquiries, please contact the following:

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