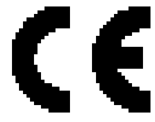
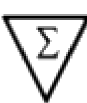


Human papillomavirus 16/18 Genotyping PCR kit

Real Time PCR test for detection of *Human papillomavirus 16, Human papillomavirus 18* DNA

Instructions for use

IVD

Human papillomavirus 16/18 Genotyping PCR kit		*112 Untersuchungen* *112 tests*	**Form S 87-04** **Form T 87-05** **Form F 87-06**	
Human papillomavirus 16/18 Genotyping PCR kit		*56 Untersuchungen* *56 tests*	**Form S 87-04-56** **Form T 87-05-56** **Form F 87-06-56**	
Human papillomavirus 16/18 Genotyping PCR kit		*24 Untersuchungen* *24 tests*	**Form S 87-04-24** **Form T 87-05-24** **Form F 87-06-24**	

1. SYMBOLS LEGEND

Symbol	Description	Symbol	Description
IVD	In-vitro Diagnostika In vitro diagnostic medical device	CE	EG-Konformitätserklärung EC Declaration of conformity
REF	Bestellnummer Catalogue number	LOT	Chargenbezeichnung Batch code
	Verwendbar bis Use by		Hersteller Manufacturer
	Herstellungsdatum Date of manufacture		Temperaturbegrenzung Temperature limitation
	Vor Licht schützen Protect from light		trocken halten Keep dry
Σ	ausreichend für <112/56/24> Tests Contains sufficient for < 112/56/24 > tests		Achtung, Begleitdokumente beachten Caution, consult accompanying documents
i	Gebrauchsanweisung beachten Consult operating instructions	HPV 16/18-Mix-T	PCR-Mix *Human papillomavirus 16/18*, Form T
NC	Negativkontrolle Negative Control	HPV 16/18-Mix-S	PCR-Mix Human *papillomavirus 16/18*, Form S
Taq	Taq-DNA-Polymerase Taq DNA-polymerase	HPV 16/18-Mix-F	PCR-Mix *Human papillomavirus 16/18*, Form F
PC	Positivkontrolle Positive Control		

1. INTENDED USE

The ***Human papillomavirus 16/18* Genotyping PCR kit** is intended for fast qualitative Real-Time PCR detection of *Human papillomavirus 16* and *Human papillomavirus 18* DNA in urethral swabs, endocervical and vaginal swabs, oropharyngeal swabs and rectal swabs samples.

2. PRINCIPLE OF THE TEST

Detection of ***Human papillomavirus 16/ Human papillomavirus 18 DNA*** includes stages of DNA extraction, PCR amplification, and data analysis.

The test is based on simultaneous amplification (genotyping-PCR) of DNA fragments of HPV and a fragment of β2-microglobulin gene (B2M) which is used as an internal endogenous control. The use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material.

Then during Real-Time PCR DNA of *Human papillomavirus 16*, *Human papillomavirus 18* and DNA B2M gene is amplified with specific primers and detected with specific hydrolysis fluorescent probes. Thermocycler detects emitted fluorescence and calculates quantification cycle Cq to determine the presence or absence of target DNA.

The “hot start” Taq DNA-polymerase used in the kit improves sensitivity and specificity of the test.

3. MATERIALS PROVIDED

3.1 Form S (reaction mix under paraffin in PCR 0.1 mL low profile 8-tube strips; Taq DNA polymerase supplied in a separate tube).

Label	Component	87-04		87-04-56		87-04-24	
		Volume, µL	Quantity	Volume, µL	Quantity	Volume, µL	Quantity
HPV 16/18-Mix-S	PCR-Mix *HPV 16/ HPV 18*, Form S	10	14×8× 0.1 mL tubes	10	7×8×0.1 mL tubes	10	3×8×0.1 mL tubes
Taq	Taq DNA-polymerase	1120	1 tube	560	1 tube	240	1 tube
PC	Positive Control	250	1 tube	250	1 tube	250	1 tube
NC	Negative Control	1500	1 tube	1500	1 tube	1500	1 tube

The Form S of the product was validated for use with the following real-time PCR cyclers: Bio-Rad CFX96 (also can be used with CFX96 Touch), Thermo Fisher Scientific/Applied Biosystems QuantStudio 5 (96-well 0.1 ml block), Roche Light Cycler 96 and DNA-Technology DTprime (corresponds to DTlite, SaCycler). To use other real-time PCR thermocyclers compatible with low profile PCR tubes, contact the manufacturer for more information concerning compatibility.

3.2 Form T (reaction mix under paraffin in PCR 0.2 mL regular profile reaction tubes; Taq DNA polymerase supplied in a separate tube).

Label	Component	87-05		87-05-56		87-05-24	
		Volume, µL	Quantity	Volume, µL	Quantity	Volume, µL	Quantity
HPV 16/18-Mix-T	PCR-Mix *HPV 16/ HPV 18*, Form T	10	112×0.2 mL tubes	10	56×0.2 mL tubes	10	24×0.2 mL tubes
Taq	Taq DNA-polymerase	1120	1 tube	560	1 tube	240	1 tube
PC	Positive Control	250	1 tube	250	1 tube	250	1 tube
NC	Negative Control	1500	1 tube	1500	1 tube	1500	1 tube

The Form T of the product was validated for use with the following real-time PCR cyclers: Qiagen Rotor-Gene Q, Corbett Research Rotor-Gene 3000/6000, and Thermo Fisher Scientific/Applied Biosystems QuantStudio 5 (96-well 0.2 mL block). To use other real-time PCR thermocyclers compatible with regular profile PCR tubes, contact the manufacturer for more information concerning compatibility.

3.3 **Form F** (liquid reaction mix is in 1.5 mL tube)

Label	Component	87-06		87-06-56		87-06-24	
		Volume, µL	Quantity	Volume, µL	Quantity	Volume, µL	Quantity
HPV 16/18-Mix-F	PCR-Mix *HPV 16/ HPV 18*, Form F	1120	1 tube	560	1 tube	240	1 tube
Taq	Taq DNA-polymerase	1120	1 tube	560	1 tube	240	1 tube
PC	Positive Control	250	1 tube	250	1 tube	250	1 tube
NC	Negative Control	1500	1 tube	1500	1 tube	1500	1 tube

The Form F of the product was validated to be used with following real-time PCR cyclers: Qiagen Rotor-Gene Q (also can be used with Corbett Research Rotor-Gene 3000/6000), Bio-Rad CFX96 (also can be used with CFX96 Touch), DNA-Technology DTprime (corresponds to DTlite, SaCycler), Thermo Fisher Scientific/Applied Biosystems QuantStudio 5 (96-well 0.1 mL block, 96-well 0.2 mL block), Roche Light Cycler 96, and Bio Molecular Systems MIC. While using other real-time PCR cyclers contact the manufacturer for more information concerning compatibility.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Biosafety cabinet;
- Real-Time PCR cycler: Qiagen Rotor-Gene Q (also can be used with Corbett Research Rotor-Gene 3000/6000), Bio-Rad CFX96 (also can be used with CFX96 Touch), DNA-Technology DTprime (corresponds to DTlite, SaCycler), Thermo Fisher Scientific/Applied Biosystems QuantStudio 5, Bio Molecular Systems MIC or Roche Light Cycler 96;
- Vortex mixer;
- Microcentrifuge for Eppendorf tubes;
- Microcentrifuge suitable for PCR 0.2 mL tubes, or PCR 0.2 mL 8-tube strips (max. 2400 rpm);
- Two separate pipette sets for DNA-free and DNA-containing components;
- Filter tips (10, 100; 200 and 1000 µL);

- Disposable safe-seal 1.5 mL microcentrifuge tubes, DNAse/RNAse free;
- Separate working areas for PCR mix preparation (Reagent preparation area), DNA extraction and PCR setup (Sample preparation area), and for Real-Time PCR amplification (Amplification area). Each area must be fitted with separate set of pipettes, instruments and laboratory clothing;
- Multi DNA extraction kit (REF 80-01), Prime DNA/RNA Extraction kit (REF 80-07), Magnetic DNA/RNA Extraction kit (REF 80-06), Magnetic DNA/RNA Auto Extraction kit (REF 80-08, 80-08-P1) or Magnetic Plus DNA/RNA Auto Extraction kit (REF 80-09, 80-09-P1).

5. STORAGE CONDITIONS AND STABILITY OF THE KIT

The kit contains consumables and reagents to perform 112 (Ref. 87-04/87-05/87-06), 56 (Ref. 87-04-56/87-05-56/87-06-56) or 24 (Ref. 87-04-24/87-05-24/87-06-24) reactions, including controls.

***Human papillomavirus 16/18* Genotyping PCR kit** should be stored at -18…-30 °C, preferably in the original kit box until the expiration date. The manufacturer guarantees the functionality of the kit components up to 15 freezing and thawing cycles.

The expiry date of the kit is shown on the box label, expiry date for each component is indicated on the respective label. Storage at +25 °C is allowed for no more than 7 days (any kit format).

6. SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Total DNA can be extracted from urethral swab specimens, endocervical and vaginal smears, oropharyngeal swabs and rectal swabs samples.

Type of specimen	Collection*	Storage-Transport**
urethral swab, endocervical and vaginal smears, oropharyngeal swabs and rectal swabs	Specimens are collected with suitable collection device and transferred and transported in 0.5 mL of 0.9 % NaCl solution or other transport medium suitable this purpose	• At +18…+25 °C for ≤ 48 hours • Refrigerated at +2…+8 °C for ≤ 4 days • Frozen at -18…-35 °C for > 4 days

*As example; use recommended standard collection procedures to obtain specimens.

**Ensure that the transportation and storage of human specimens meets all local and national regulations for the transport of infectious agents.

7. ASSAY PROCEDURE

The complete procedure consists of three stages:

I. DNA Extraction
II. Real-Time PCR
III. Data analysis and interpretation

I. DNA extraction

NOTE: *The quality of the extracted DNA has a profound impact on the performance of the entire test system. Ensure that the system used for DNA extraction is compatible with real-time PCR technology.* ***Human papillomavirus 16/18*** **Genotyping PCR kit** *has been validated with the following kits and systems for DNA extraction, and they are recommended by the manufacturer:*

- *Multi DNA Extraction kit (Astra Biotech, Ref 80-01);*
- *Prime DNA/RNA Extraction kit (Astra Biotech, Ref 80-07);*
- *Magnetic DNA/RNA Extraction kit (Astra Biotech, Ref 80-06);*
- *Magnetic DNA/RNA Auto Extraction kit (Astra Biotech, REF 80-08, 80-08-P1);*
- *Magnetic Plus DNA/RNA Auto Extraction kit (Astra Biotech, REF 80-09, 80-09-P1).*

The compatibility of other DNA extraction procedures for use with Human papillomavirus 16/18 Genotyping PCR kit *has to be validated by the user.*

1) Prepare and label required quantity of 1.5 mL microcentrifuge tubes equal to the number of samples + 1 tube for negative extraction control labelled as “NEC”.

NOTE: *You do not need to put in each sample Internal Control IC, because test system uses endogenous internal control (transcript of one of human «housekeeping genes»).*

NOTE: *Each extraction procedure should be performed using “NEC”.*

2) Add **100 µL NC into** the microcentrifuge **tube marked “NEC**”.

NOTE: *The Elution buffer or Negative control contained in DNA/RNA extraction kit can be used instead of negative extraction control.*

3) Follow the manual of DNA extraction kit.

I. Real-Time PCR

IMPORTANT: Positive and Negative Controls must be included in each run.

Form S/T

Kit components preparation

NOTE*: Kit components preparation has to be done in the Reagent Preparation Area*

1) Take out from the freezer and thaw the following components of the kit:

- **PC** (positive control);
- **NC** (negative control);
- **Taq** (Taq polymerase);
- Required quantity of 8-tube strips or individual PCR tubes with **HPV16/18-Mix-S/T** for tested samples, plus 1 tube for “NEC” and 1 tube for “PC”.

The rest of components put back in the freezer.

2) Transfer **PC** and **NC** to the Sample Preparation Area.

PCR mix preparation:

***NOTE**: PCR mix preparation has to be done in the Reagent Preparation Area. Do not prepare the PCR mix in the same area as the DNA/RNA extraction or PCR amplification to prevent contamination.*

1) Label prepared **HPV 16/18-Mix-S/T** PCR tubes.

***NOTE:** When using real-time PCR cyclers with fluorescent detection through the tube cap, label tubes on the edge of the cap or on side wall. When using real-time PCR cyclers with fluorescent detection through the side wall, label tubes on the cap.*

2) Gently vortex **Taq**, avoiding foaming, and centrifuge at 1500-2400 rpm for 2-3 s.

3) Place 10 µL of **Taq** into each tube avoiding tip contact with paraffin.

Dispensing of test samples and the controls:

***NOTE**: Dispensing of test samples and controls has to be performed in the Sample Preparation Area.*

***NOTE**: Use a separate filter tip for each DNA sample.*

1) Centrifuge the DNA extracted from the specimens and “NEC” (**NC** extract) at 13000 rpm for 1 min before adding them to the PCR tubes.

2) Add 10 µL of DNA extracted from specimens and “NEC” to corresponding PCR tubes avoiding tip contact with paraffin.

3) Vortex and centrifuge the **PC** at 1500-2400 rpm for 2-3 s.

4) Add 10 µL of **PC** to the PCR tube marked “PC”.

5) Close all tubes and centrifuge at 1500-2400 rpm for 2-3 s.

Real-Time PCR Amplification:

***NOTE**: Real-Time PCR Amplification has to be performed in the Amplification area. Separate room for this area is recommended. Follow the manufacturer instruction before using PCR Real-Time cycler.*

- Load tubes into a Real-Time PCR cycler.
- Using cycler’s software, fill in the location of the samples. Set up fluorescent detection on the channels ROX/ Orange/ Texas Red, HEX/ Yellow and FAM/ Green.
- Run the PCR protocol.

<table>
<tr><th>Stage</th><th>Temperature, °C</th><th>Time</th><th>Data collection</th><th>Number of cycles</th></tr>
<tr><td>Hold</td><td>94</td><td>3 min</td><td>-</td><td>1</td></tr>
<tr><td rowspan="2">Cycling</td><td>94</td><td>10 s</td><td>-</td><td rowspan="2">5</td></tr>
<tr><td>60</td><td>20 s</td><td>-</td></tr>
<tr><td rowspan="2">Cycling</td><td>94</td><td>10 s</td><td>-</td><td rowspan="2">45</td></tr>
<tr><td>60</td><td>20 s</td><td>ROX/ Orange/ Texas Red
HEX/ Yellow
FAM/ Green</td></tr>
</table>

Form F

Kit components preparation

***NOTE:** Kit components preparation has to be done in the Reagent Preparation Area*

1) Take out from the freezer and thaw the following components of the kit:

- **PC** (positive control);
- **NC** (negative control);
- **Taq** (Taq polymerase with dNTPs);
- **HPV16/18-Mix-F** (PCR buffer, primers and probes).

2) Transfer **PC** and **NC** to the Sample Preparation Area.

PCR mix preparation:

***NOTE**: PCR mix preparation has to be performed in the Reagent Preparation Area. Do not prepare the PCR mix in the same area as the DNA/RNA extraction or PCR amplification to prevent contamination.*

1) Prepare and label required quantity of 0.1-0.2 mL PCR tubes/strips/microwell plates for tested samples, plus 1 tube/well for “NEC” and 1 tube/well for “PC”.

***NOTE:** when using real-time PCR cyclers with fluorescent detection through the tube cap, label tubes on the edge of the cap or on side wall. When using real-time PCR cyclers with fluorescent detection through the side wall, label tubes on the cap.*

2) Gently vortex **Taq** tube and centrifuge at 1500-2400 rpm for 2-3 s.

3) Vortex and centrifuge the **HPV 16/18-Mix-F** at 1500-2400 rpm for 2-3 s.

4) To prepare Master Mix, in 1.5-2 mL eppendorf tube, mix 10*N µL of **HPV 16/18-Mix-F** and 10*N µL of **Taq**, where N is the total number of reactions.

For example, to run 10 samples, and «PC» and «NEC» (12 reactions total) you should mix 120 µL of **HPV 16/18-Mix-F** and 120 µL of **Taq**.

5) Vortex prepared Master Mix and spin briefly to remove drops from the inside of the lid.

NOTE: *Do not store Master Mix (***HPV 16/18-Mix-F*** plus ***Taq***) for more than 8 hours at +2…+8 °C.*

6) Put 20 µL of Master Mix into each PCR tube/ well.

Dispensing of test samples and the controls:

NOTE*: Dispensing of test samples and controls have to be performed in the Sample Preparation Area.*

NOTE*: Use a separate filter tip for each DNA sample.*

1) Centrifuge the DNA extracted from the specimens and “NEC” (**NC** extract) at 13000 rpm for 1 min before adding them to the PCR tubes.

2) Add 10 µL of DNA extracted from specimens and “NEC” to corresponding PCR tubes avoiding tip contact with paraffin.

3) Vortex and centrifuge the **PC** at 1500-2400 rpm for 2-3 s.

4) Add 10 µL of **PC** to the PCR tube marked “PC”.

5) Close all tubes and centrifuge at 1500-2400 rpm for 2-3 s.

Real-Time PCR Amplification:

NOTE*: Real-Time PCR Amplification has to be performed in the Amplification area. Separate room for this area is recommended. Follow the manufacturer instruction before using the PCR Real-Time cycler.*

- Load tubes into a Real-Time PCR cycler.
- Using cycler’s software, fill in the location of the samples. Set up fluorescent detection on the channels ROX/ Orange/ Texas Red, HEX/ Yellow and FAM/ Green.
- Run the PCR protocol.

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<tr><td>60</td><td>20 s</td><td>ROX/ Orange/ Texas Red
HEX/ Yellow
FAM/ Green</td></tr>
</table>

III. Data Analysis

1. General information:

Analysis of the results can be performed with Real-Time PCR thermocycler data analysis software according to its instructions for use.

ROX dye is used to detect the *Human papillomavirus 16* DNA, HEX dye is used to detect the *Human papillomavirus 18* DNA and FAM dye is used to detect the DNA internal control (B2M gene).

Analysis of the results can be performed only by using parameters Cq **HPV16**, Cq **HPV18**, Cq **PC** and Cq **IC**, written in the Quality control Sheet provided with each kit, and that are specific for each lot.

Cq **HPV16** critical value of quantification cycle for *Human papillomavirus 16* in the ROX channel, that is crucial for detection positive results;

Cq **HPV18** critical value of quantification cycle for *Human papillomavirus 18* in the HEX channel, that is crucial for detection positive results;

Cq **PC** critical value of quantification cycle for Positive Control (PC) in the ROX and HEX channel is used to determine whether kit PCR amplification and detection components work properly (general control of the reagents effectiveness), that is crucial for detection negative results;

Cq **IC** critical value of quantification cycle for Internal Control (IC) in the FAM channel is used to control all stages of the analysis – quality of DNA extraction, PCR

amplification and detection (overall effectiveness) in each tube, including inhibition and DNA loss.

It is also important to consider the appearance of the amplification curve for each fluorophore detection channel – it must be of a distinctive S-shape, which indicates that amplification has proceeded correctly.

2. Determination of validity of test run results:

The amplification run is **valid**, if both of the following conditions are met:

(1) For a "PC" reaction with positive control **PC**, Cq PC ≤ Cq **PC** for both ROX and HEX channels, and all amplification curves have a distinctive S-shape;
(2) For a "NEC" reaction with negative control **NC**, Cq NEC > Cq **IC** in the FAM channel, Cq NEC > Cq **HPV16** in the ROX channel, Cq NEC > Cq **HPV18** in the HEX channel, or are not determined.

If one of these conditions is not met, the amplification run is considered **invalid**, and test samples should not be analysed.

NOTE for (1): For a "PC" reaction with positive control **PC**, if Cq PC > Cq **PC**, then all specimens defined as positive in this test run should be considered as positive, whereas all the specimens defined as negative should be considered as invalid.

NOTE for (2): For a "NEC" reaction with negative control **NC**, Cq NEC ≤ Cq **HPV16** in the ROX channel and/or Cq NEC ≤ Cq **HPV18** in the HEX channel indicates possible contamination. Measures must be taken to find and eradicate the source of

contamination. In case of contamination, all positive specimens satisfying condition Cq ≥ Cq (NEC – 5) in the ROX or/and HEX channel are considered as invalid, the other results are considered as valid.

3. Determination of sample status in test run:

We recommend to analyze each sample for presence of *Human papillomavirus 16/ Human papillomavirus 18* separately according to following tables. All amplification curves with determined Cq must be of distinctive S-shape:

PC reaction	NEC reaction	Test sample for HPV16 (ROX)	Sample status
Valid test run	Valid test run	Cq ≤ Cq **HPV16**	Positive
Valid test run	Valid test run	Cq > Cq **HPV16** or N/A	Negative
Cq PC > Cq **PC**	Valid test run	Cq ≤ Cq **HPV16**	Positive
Cq PC > Cq **PC**	Valid test run	Cq > Cq **HPV16** or N/A	Invalid
Valid test run	Cq NEC ≤ Cq **HPV16**	Cq < Cq (NEC-5)	Positive
Valid test run	Cq NEC ≤ Cq **HPV16**	Cq ≥ Cq (NEC-5)	Invalid

PC reaction	**NEC reaction**	**Test sample for HPV18 (HEX)**	**Sample status**
Valid test run	Valid test run	Cq ≤ Cq **HPV18**	Positive
Valid test run	Valid test run	Cq > Cq **HPV18** or N/A	Negative
Cq PC > Cq **PC**	Valid test run	Cq ≤ Cq **HPV18**	Positive
Cq PC > Cq **PC**	Valid test run	Cq > Cq **HPV18** or N/A	Invalid
Valid test run	Cq NEC ≤ Cq **HPV18**	Cq < Cq (NEC-5)	Positive
Valid test run	Cq NEC ≤ Cq **HPV18**	Cq ≥ Cq (NEC-5)	Invalid

Abbreviations:

Cq **HPV16** *critical value of quantification cycle for Human papillomavirus 16;*

Cq **HPV18** *critical value of quantification cycle for Human papillomavirus 18;*

Cq **IC** *critical value of quantification cycle for Internal Control (IC);*

Cq **PC** *critical value of quantification cycle for Positive Control (PC).*

Cq **HPV16**, Cq **HPV18**, Cq **IC**, Cq **PC** are specified for each lot in the Quality control Sheet provided with each kit.

NEC	Negative Extraction Control
Cq of NEC	*quantification cycle for NEC in the ROX, HEX channel is determined in case of contamination*
N/A	*Cq value is not determined*

8. PERFORMANCE CHARACTERISTICS OF THE KIT

Analytical sensitivity:

The detection limit of the kit as evaluated via probit analysis is 1000 copies of *HPV 16 and* 1000 copies *HPV 18* genomic DNA per 1 mL. This limit is defined for confidence level 95 %. It is assumed that 100 µL of specimen was sampled for DNA extraction and elution volume was 60 µL.

Analytical specificity:

The specificity of the oligonucleotides were tested *in silico* on following organisms: *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genitalium, Gardnerella vaginalis, Lactobacillus spp, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Trichomonas vaginalis, Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, Neisseria gonorrhoeae, Neisseria flava, Neisseria subflava, Neisseria mucosa, Neisseria sicca, Treponema pallidum, Toxoplasma gondii, Candida albicans, HSV types 1 and 2, HPV 31 33 35 39 45 51 52 58 56 59 66 68, Gardnerella vaginalis, HHV 6, HHV 7, HHV 8, Varicella zoster virus, Epstein–Barr virus, Cytomegalovirus*. To perform this test NCBI BLAST service was used.

9. LIMITATIONS OF THE METHOD

Any clinical diagnosis should not be based on the results of *in vitro* diagnostic methods alone. For final diagnosis a physician is supposed to consider all available clinical and laboratory tests findings.

10. SAFETY PRECAUTIONS

This kit is for in vitro diagnostic use only. The operator should thoroughly follow the manual to obtain reliable data. This instruction manual is valid only for the present kit with the listed contents. Any exchange of the kit components is not allowed by CE regulations.

- Test should only be performed by skilled personnel considering GLP (Good Laboratory Practice) guidelines.
- Do not use the kit after its expiration date.
- Do not pool reagents from different lots or from different tubes of the same lot (the only exception for **IC** is described below). Immediately after use, close all tubes in order to avoid leakage.
- In case of simultaneous using of two or more Astra Biotech Infectious Real-Time PCR kits for detection of different infectious agents, **IC** with the latest lot number can be used for all of these kits.
- We recommend setting up blank protocols of PCR before the analysis.
- PCR technology is extremely sensitive. The amplification of a single DNA molecule generates millions of identical copies. Therefore, set up three separate working areas for a) sample

preparation b) PCR reagent preparation and c) PCR amplification. For each working area a different set of pipettes and protective clothing should be reserved.

- Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting.
- Do not use the same tip for two different components, neither DNA-free nor DNA containing.
- Routinely decontaminate your pipettes and the laboratory benches.
- After usage, all the reagents and test components should be discarded as consisted with local statements.

Any materials of animal origin used for kit components preparation were found safe for humans. However, none of known laboratory test guarantees total absence of the hazard agents. Therefore, all kit components and patient’s samples should be handled as potentially hazardous.

After usage the kit components, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving or disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.

The following precautions should be taken:

- do not smoke, eat or drink while performing the assay;
- always use protective gloves while performing the assay;

- never pipette material by mouth;
- be careful while handling **PC**, avoid its splashing;
- in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.
- GLP including all general and individual regulations should be applied for the kit usage.

11. REFERENCE

Burd EM. Human Papillomavirus Laboratory Testing: the Changing Paradigm. Clin Microbiol Rev. 2016 Apr;29(2):291-319. doi: 10.1128/CMR.00013-15. PMID: 26912568; PMCID: PMC4786885.

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