

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
RUO	Research Use Only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer		Negative control of extraction
	Date of manufacture		Positive control of amplification
			C1, C2 DNA-calibrators

1. INTENDED USE

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit is not a medical device. PCR kit is intended for quantitative detection of *human papillomaviruses* of high carcinogenic risk (HPV HCR) DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 in the biological material (urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix)) using real-time hybridization-fluorescence detection of amplified products. The PCR kit allows to determine the total number of HPV DNA of all 14 types and also to separately identify the HPV DNA of types 16, 18 and 45. The material for PCR is the DNA samples extracted from the test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material obtained during screening studies or taken from persons with suspected papillomavirus infection, without distinction of form and presence of disease manifestation. There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material and the simultaneous amplification of DNA fragments of the detected HPV HCR types and DNA of the human β -globin gene with hybridization-fluorescence detection. DNA of the β -globin gene is used as an endogenous internal control (IC Glob) and allows not only to control all stages of the PCR study for each sample, but also to evaluate the adequacy of the material and its storage. IC Glob, being a part of the human genome, should always be present in the test sample in a sufficient amount equivalent to the number of human cells in the sample ($500 \cdot 10^5$ cells/reaction).

Amplification of DNA fragment with the use of specific primers and Taq-polymerase enzyme is performed with the DNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Quantitative analysis of HPV HCR DNA is based on the linear dependence between the initial concentration of DNA target in a test sample and the cycle threshold (Ct) (the cycle of beginning of fluorescence signal exponential growth). Quantitative analysis is performed in the presence of DNA calibrators (samples with a known concentration of DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of the concentration of the DNA target in test samples. HPV DNA concentration is calculated as the relation between number of HPV copies and number of epithelial cells of human membrane mucosa.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP.

The results of amplification are registered in five different fluorescence channels:

Channel for fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
DNA target	DNA of HPV HCR type 16	DNA of HPV HCR type 18	types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	DNA fragment of β -globin gene (IC Glob)	DNA of HPV HCR type 45
Target gene	E6 gene	E6 gene	E1 gene (for types 16, 31, 33, 35, 52, 58)/ E2 gene (for types 18, 39, 45, 56, 59, 66, 68)/ E7 gene (for type 51)	β -globin gene	E6 gene

HPV types 16, 18 and 45 in conjunction are known to cause around 75% squamous cell carcinomas and 94% cervical adenocarcinoma cases. Moreover, the development of cervical cancer is often associated with the integration of HPV DNA into the genome of the host cell, which results in a break in the E1/E2 region while maintaining the E6/E7 oncogene.

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit has been developed to detect different genome section of HPV types 16, 18 and 45 in the different detection channels (E6 region – in the channels for FAM, JOE, Cy5.5 fluorophores, E1/E2 – in the channel for ROX fluorophore). Detection of E6 region (in the absence of E1/E2 region) allows indirectly to judge about the possibility of virus integration into the human genome.

3. CONTENT

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit is produced in 2 forms:

variant FRT-100 FN. H-2311-1-13-CE;

variant FRT-100 FN in bulk¹. H-2311-1-13-CE-B.

Variant FRT-100 FN includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL HPV 14	clear liquid from colorless to grey-blue colour	1.2	1 tube
PCR-buffer-H	colorless clear liquid	0.6	1 tube
DNA calibrator C1 HPV screen	colorless clear liquid	0.2	1 tube
DNA calibrator C2 HPV screen	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	2 tubes

* must be used in the extraction procedure as Negative Control of Extraction (see DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO, AmpliSens® DNA-sorb-D protocols).

Variant FRT-100 FN is intended for 110 reactions, including controls.

AmpliSens® HPV HCR screen-titre-14 software in Microsoft® Excel format for data processing and result generation.

4. ADDITIONAL REQUIREMENTS

Sampling and pretreatment

- Transport medium.
- Cervical sampler.
- Endocervical brush.
- Gynaecological combined probe.
- Disposable polypropylene tightly closed 1.5; 2-ml 5.0-ml tubes.
- Sterile RNase-free pipette tips up to 200 μ l.
- Sterile RNase-free pipette tips with filters up to 1000 μ l.
- Tube racks.
- PCR box.
- Vortex mixer.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Vacuum aspirator with flask for removing supernatant.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

For DNA extraction and amplification

- DNA extraction kit.
- Set of consumables for used automated station according to the manufacturer's manual for the DNA extraction reagent kit.
- Disposable polypropylene PCR tubes:
 - a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation;
 - b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
 - c) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Sterile RNase-free pipette tips up to 100 μ l, 200 μ l, 1000 μ l.
- Tube racks.
- PCR box.
- Vortex mixer.
- Pipettes (adjustable).
- Real-time instruments (for example, Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), CFX96 (Bio-Rad, USA)).
- Refrigerator with the range from 2 to 8 °C.
- Deep-freezer with the range from minus 24 to minus 16 °C.
- Disposable powder-free gloves and laboratory coat.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse

¹ In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in the DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where the previous step was performed.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material:

- vaginal swab,
- epithelial scrape from the cervical mucous membrane (ectocervix and endocervix).

Sampling

Vaginal swab

The material should be obtained from the posterolateral fornix of the vagina using a swab or a combined probe into a test tube with 0.5 ml of **Transport Medium with Mucolytic Agent**. Rotate the working part of the probe on the surface of the side walls of the vagina, collecting the discharge as completely as possible. Minimal presence of impurities such as mucus and blood is acceptable. Place the swab in the test tube with transport medium. Break off the working part of the probe with the test material and leave it in the test tube with the transport medium. Tightly close the test tube, avoiding a gap and crumpling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

In case of using **Transport Medium with Mucolytic Agent** its colour can be changed at acidic pH of discharge.

Samples of the test material can be stored before the PCR-analysis:

- at the temperature from 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - within 3 months;
- at the temperature from minus 20 °C and below - for a long time.

Only one freeze-thawing cycle is required.

Epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix)

Use a disposable or reusable gynaecological speculum to access to the cervical canal. Before obtaining the material, remove mucus and vaginal discharge from the surface of the cervix with a sterile gauze swab (minimal presence of impurities such as cervical mucus and blood is acceptable). Take the material using an endocervical brush (cytobrush) or a combined gynaecological probe (it is allowed to use for pregnant women, young nulliparous women).

Methods of taking epithelial scrapes from the mucous membrane of the cervical canal (ectocervix and endocervix)

Method 1 - One/two cytobrushes and a tube with 0.5 ml of **Transport Medium with Mucolytic Agent** are used. Place the cervical epithelial scrape (endocervix) taken with the first cytobrush and/or the superficial cervical scrape (ectocervix) taken with the second cytobrush to the tube with transport medium.

Method 2 - DNAPAP Cervical Sampler (QIAGEN GmbH, Germany), containing a cytobrush and a tube with **digene Specimen Transport Medium** is used. Place the cervical epithelial scrape (endocervix) into the tube with **digene Specimen Transport Medium**.

Method 3 - a combined gynaecological probe for simultaneous collection of epithelium from endocervix and ectocervix and 5-ml tube with 2 ml of **Transport Medium with Mucolytic Agent** are used. Place the cervical epithelial scrape (endocervix and ectocervix) into the tube with transport medium.

Method 4 - a combined gynaecological probe for simultaneous collection of epithelium from endocervix and ectocervix and a vial with transport-fixing medium for liquid-based cytology are used. Place the cervical epithelial scrape (endocervix and ectocervix) into the vial with transport medium.

Break off the working part of the cytobrush/probe with the test material and leave it in the tube or vial with the transport medium. Tightly close the tube/vial with a cap, avoiding gaps and wrinkling of the inner part of the cap. If it is impossible to break off the working part of the cytobrush/probe it should be immersed in the transport medium and pressed against the inner side of the tube/vial. Rotate for 5–10 s, then remove the cytobrush/probe and tightly close the test tube/vial.

It is not allowed to use scissors to cut the working part of the probe!

The samples of test material taken in **Transport Medium with Mucolytic Agent** can be stored before the PCR-analysis:

- at the temperature from 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - for 3 months;
- at the temperature below minus 20 °C and - for a long time.

Only one freeze-thawing cycle is required.

The samples of test material taken in the **digene Specimen Transport Medium** can be stored before the PCR-analysis:

- at a temperature of minus 20 °C - for 3 years.

Three freeze-thawing cycle are required.

The samples of test material taken in the transport-fixing medium for liquid-based cytology can be stored before pretreatment:

- at the temperature of 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - for 6 months.

Pretreatment

Pretreatment for **vaginal swabs and epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix) taken in Transport medium with mucolytic agent or digene Specimen transport medium** is not required.

Scraping samples taken in transport-fixing alcohol-containing medium for liquid cytology are to be pretreated (epithelial cell concentration).

Epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix) taken in transport-fixing alcohol medium for liquid-based cytology

Take An aliquot of cells for the PCR analysis only with disposable filter tips and only into disposable sterile tubes. It is important to take an aliquot of cells first for the PCR analysis and then for liquid-based cytology.

NOTE:

Epithelial cell concentration

Method 1

Vigorously shake the vial with sample for liquid cytology to disintegrate cells and leave it at 18-25°C for 30 min to sediment cells. Then transfer 1 ml of the precipitate into a 1.5 ml tube using a filter tip. Centrifuge for 5 min at 12,000 g (e.g., 13,400 rpm for a MiniSpin microcentrifuge). Without disturbing the precipitate, remove the supernatant using a non-filter tip and vacuum aspirator, leaving 100 µl of supernatant and precipitate. Thoroughly mix on a vortex and sediment drops from the tube walls. Use the obtained sample for DNA extraction, starting from washing concentrated epithelial cells.

Method 2

Vigorously shake the vial with the sample for liquid-based cytology to disintegrate the cells. Transfer 1-2 ml of the cell suspension into a 2-ml tube using a filter tip. Centrifuge for 5 min at 12,000 g (e.g., 13,400 rpm for a MiniSpin microcentrifuge). Without disturbing the precipitate, remove the supernatant using a non-filter tip and vacuum aspirator, leaving 100 µl of supernatant and precipitate. After thorough mixing on a vortex and drops sedimentation from the walls of the tube and the inside of the lid by centrifugation for 3-5 s, use the obtained sample for DNA extraction, starting from washing of concentrated epithelial cells step.

Interfering substances and limitations of using test material samples

Samples containing no more than 20 % of potentially interfering substances are applicable for analysis.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 2).

Model samples of urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix)) without adding and with the addition of potentially endogenous and exogenous potential interfering substances were tested. Concentration of each potential interfering substance in a sample is specified in Table 2. Model samples contained quality control samples (QCS) with HPV HCR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 with concentrations of each target 5x10³ copies/ml, equal to the detection limit.

Table 2

Type of tested material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix))	Endogenous substances	Mucus	20 %	Not detected
		Whole blood	20 %	Not detected
	Exogenous substances	Sperm	20 %	Not detected
		Nystatin	20 %	Not detected
		Lubricant	20 %	Not detected
		Miramistin	20 %	Not detected

7. WORKING CONDITIONS

AmpliSens® HPV HCR screen-titre-14-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA Extraction

Reagent kits for DNA extraction from different types of test material, volumes of the test sample, negative control of extraction (C-) and elution are specified in Table 3. **Negative Control (C-)** reagent is used as a Negative control of extraction:

Table 3

Test material	Transport medium	Nucleic acid extraction kit	Test sample volume, µl	Negative Control of Extraction (C-) volume, µl	Elution volume, µl
urogenital mucous discharge (vaginal swab)	Transport Medium with Mucolytic Agent	DNA-sorb-AM	100	100	100
		AmpliSens® MAGNO-sorb-URO	100	100	100
urogenital mucous discharge (epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix))	Transport Medium with Mucolytic Agent, digene Specimen Transport Medium	DNA-sorb-AM	100	100	100
		AmpliSens® MAGNO-sorb-URO	100	100	100
	Transport medium for liquid-based cytology	AmpliSens® DNA-sorb-D	1000*	100	100
		AmpliSens® MAGNO-sorb-URO	1000*	100	100

*Refer to this Instruction manual and the instruction manuals for the nucleic acid extraction kit for the volume and procedure of preparation of the test material.

NOTE: Extract DNA according to the manufacturer's protocol.

NOTE: Addition of Internal control (IC) is not required.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

The type of tubes depends on the type of PCR real-time instrument. Use disposable tips for adding reagents, DNA and control samples into tubes. The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

1. Calculate the volume of each reagent required for reaction mixture preparation. For one reaction:

- 10 µl of PCR-mix-FL HPV 14
- 5 µl of PCR-buffer-H.

Prepare the reaction mixture for the total number of test and control samples (see item 7 for the number of control samples) plus extra volume for several reactions.

NOTE: Reaction mixture components should be mixed just before analysis.

- Thaw the tube with **PCR-mix-FL HPV 14**. Vortex the tubes with **PCR-mix-FL HPV 14**, **PCR-buffer-H** and sediment the drops.
- To prepare the reaction mixture, mix the required quantity of **PCR-mix-FL HPV 14** and **PCR-buffer-H** in a new sterile tube. Sediment the drops on vortex.
- Take the required number of tubes/strips for amplification of the DNA of test and control samples.
- Transfer 15 µl of the prepared mixture to each tube. Discard the unused reaction mixture.

6. Add **10 µl of DNA samples** obtained at the DNA extraction stage from test samples to the prepared tubes.
- NOTE:** Avoid transferring sorbent beads together with the DNA sample in case of extraction using reagent kit with sorption on silica gel or magnetic separation.
7. Carry out the control amplification reactions:
- C1** – Add **10 µl of DNA calibrator C1 HPV screen** to the tube with reaction mixture
- C2** – Add **10 µl of DNA calibrator C2 HPV screen** to the tube with reaction mixture
- C-** – Add **10 µl of the sample extracted from the Negative Control (C-) reagent** to the tube with reaction mixture
- NOTE:** It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination.
- NCA** – Add **10 µl of TE-buffer** to the tube with reaction mixture

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows (Tables 4, 5)² and run the amplification program with fluorescence detection:

Table 4

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	20 s	FAM, JOE, ROX, Cy5, Cy5.5	

NOTE: Any combination of the tests including test with reverse transcription and amplification can be performed in one instrument simultaneously with the use of the unified amplification program. If only the tests for pathogen agent DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be omitted for time saving.

Table 5

Step	Rotor-type instrument ³			Plate-type instrument ⁴		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
	95	5 s		95	5 s	
2	60	20 s	5	60	20 s	5
	72	15 s		72	15 s	
	95	5 s		95	5 s	
3	60	Fluorescence acquiring	40	60	30 s Fluorescence acquiring	40
	72	15 s		72	15 s	
	95	5 s		95	5 s	

Fluorescent signal is detected in the channels for the **FAM, JOE, ROX, Cy5.5** and **Cy5** fluorophores.

NOTE: **AmpliSens-1** is an universal program for conducting tests for identifying *human papillomaviruses* (HPV HCR) and detection of STIs and other infections of reproductive system with AmpliSens PCR kits. Therefore, any combination of these tests can be carried out simultaneously in the same instrument.

9. DATA ANALYSIS

The curves of fluorescent signal accumulation indicating the amplification product accumulation are analyzed in five channels:

Table 6

Channel for the fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
Amplification product	DNA of HPV type 16	DNA of HPV type 18	DNA of HPV HCR (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	IC β-globin DNA	DNA of HPV type 45

Analysis and interpretation of the results obtained are performed using the software of the instrument used for PCR with real-time detection using the algorithm below or the AmpliSens[®] HPV HCR-screen-titre-14 software based on Microsoft[®] Excel.

Operation of the AmpliSens[®] HPV HCR-screen-titre-14 software is carried out using the Microsoft[®] Excel program included in Microsoft[®] Office applications. To start work it is necessary to copy the file of the software from the media or the Manufacturer's website to the hard disc of the personal computer. The procedure of work with the software, as well as its purpose and characteristics are described in the "Instructions" tab of the AmpliSens[®] HPV HCR-screen-titre-14 software.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value of the DNA sample in the corresponding column of the results grid.

The calibration curve is automatically plotted on the basis of obtained threshold Ct values and known calibrators (C1 and C2) values, and human DNA and HPV DNA concentrations (copies/reaction) are calculated. Obtained values are used for calculation of HPV DNA quantity per 1x10⁵ human cells. Normalized values reflect the number of copies of the pathogen relative to human cells. Moreover the concentration values of human DNA allows to estimate the quality of biological material sampling.

For the quantitative study, a calibration line is automatically plotted based on the specified DNA calibrators' concentration values and the obtained Ct values. The obtained data is used to calculate the concentration values of the HPV HCR types to be detected.

The results of the analysis is considered reliable only if the results obtained for controls of amplification and extraction stages are correct (according to Table 7 and the Important Product Information Bulletin enclosed to the PCR kit).

Results for controls

Table 7

Control	Stage for control	Ct value in the channel for fluorophore				
		FAM	JOE	ROX	Cy5	Cy5.5
C-	DNA extraction	Absent	Absent	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent	Absent	Absent
C1	PCR	Defined	Defined	Defined	Defined	Defined
C2	PCR	Defined	Defined	Defined	Defined	Defined

Principle of interpretation of the results is specified in table 8.

Calculation algorithm:

- 1 Calculation of the number of HPV HCR DNA and human DNA (IC Glob) copies per reaction of the test and control samples using the plotted calibration line.
- 2 Calculation of the number of HPV HCR DNA copies in 1 ml of the test sample (absolute concentration) according to the formula:

$$\text{number of HPV DNA copies per reaction} \times 100 = \text{copies/ml}$$

- 3 Calculation of the amount of HPV HCR DNA per 1x10⁵ human cells (relative concentration), according to the formula:

$$\lg \left(\frac{\text{number of HPV DNA copies per reaction}}{\text{number of human DNA copies per reaction}} \right) \times 2 \cdot 10^5 = \lg (\text{HPV DNA copies} / 10^5 \text{ human cells})$$

NOTE: The relative (normalized) concentration reflects the amount of the pathogen relative to human cells. In addition, the value of human DNA concentration allows us to assess the quality of the collection of biological material.

When calculating the total amount of HPV DNA should be taken into account, that individual concentrations of HPV types 16, 18 and 45 are calculated at FAM, JOE, Cy5.5 channels.

NOTE: Concentration values of calibrators are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

For the subsequent runs with the given lot of the **AmpliSens[®] HPV HCR screen-titre-14-FRT** PCR kit one can use the results of DNA calibrator C1 obtained in the previous run on this instrument. For that purpose export the results of DNA calibrator C1 using the software of the instrument. In this case the run only for the DNA calibrator C1 is required.

Table 8

Results interpretation for the test samples

Result	Interpretation
Invalid	DNA concentration of IC Glob is less than 1x10 ³ copies/reaction (500 cells/reaction) and the calculated concentration values of HPV HCR DNA are absent in the channels for FAM, JOE, ROX, Cy5.5 fluorophores. It is necessary to repeat the PCR analysis of this sample starting from DNA extraction stage. If IC Glob DNA is absent in the test sample, it is recommended to repeat biological material sampling and PCR-analysis
HPV HCR DNA is not detected	The Ct value for HPV HCR DNA is absent and the concentration of IC Glob is more than 1x10 ³ copies/reaction (500 cells/reaction)
<3 lg (HPV per 10 ⁵ human cells)	Clinically insignificant value
3–5 lg (HPV per 10 ⁵ human cells)	Clinically significant value. Dysplasia cannot be excluded; risk of dysplasia development
>5 lg (HPV per 10 ⁵ human cells)	Clinically significant, increased value. High probability of dysplasia
Integration? (only for 16, 18 and 45 types)	Identification of E6 area in the absence of E1/E2 area indirectly suggests the probability of viral integration into the human DNA.

If the absolute concentration of HPV HCR DNA in the test sample falls out of the measurement range of the PCR kit (see Table 8) or the concentration of IC Glob DNA is less than 1x10³ copies/reaction (500 cells/reaction), the relative concentration is not calculated, the clinical significance is not determined.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The Ct value is determined for the Negative Control of Extraction (C-) in the channels for the FAM and/or JOE and/or ROX and/or Cy5 and/or Cy5.5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples in which specific DNA was detected.
2. The Ct value is determined for the Negative Control of Amplification (NCA) in the channels for the FAM and/or JOE and/or ROX and/or Cy5 and/or Cy5.5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The extraction and detection should be repeated for all samples in which specific DNA was detected.
3. The Ct value is absent for the DNA-calibrators C1, C2 in any detection channel (see Table 7). It is necessary to repeat the amplification and detection for all the samples.
4. The efficiency E is less than 80 % or greater than 120 % when plotting the calibration curve. Check the correctness of set concentrations of DNA-calibrators in accordance with the *Important Product Information Bulletin* and the correctness of selected level of the threshold line. If set concentrations of DNA-calibrators and the threshold line level are correct but the efficiency does not fit in the required range, then the amplification and detection for all the samples should be repeated.
5. If the Ct value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11. TRANSPORTATION

AmpliSens[®] HPV HCR screen-titre-14-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days

12. STABILITY AND STORAGE

All components of the **AmpliSens[®] HPV HCR screen-titre-14-FRT** PCR kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens[®] HPV HCR screen-titre-14-FRT** PCR kit are stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: PCR-mix-FL HPV 14 is to be kept away from light

² The amplification programs (Tables 4, 5) are equivalent in use for this PCR kit.

³ For example, Rotor-Gene 6000 (Corbett Research), Rotor-Gene Q (QIAGEN, Germany).

⁴ For example, CFX96 (Bio-Rad, USA).

13. SPECIFICATIONS

13.1. Analytical sensitivity and linear range

Table 9

Biological material	Transport medium	DNA extraction kit	Analytical sensitivity, copies/ml ⁵	Linear measurement range, copies/ml
Urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix))	Transport Medium with Mucolytic Agent or digene Specimen Transport Medium	DNA-sorb-AM, AmpliSens [®] MAGNO-sorb-URO	5x10 ³	7x10 ³ – 1x10 ⁸
	Transport medium for liquid-based cytology	AmpliSens [®] DNA-sorb-D, AmpliSens [®] MAGNO-sorb-URO		

The claimed features are achieved while respecting the rules specified in the section *Sampling and Handling*.

13.2. Analytical specificity

The analytical specificity of AmpliSens[®] HPV HCR screen-titre-14-FRT PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects *Chlamydia trachomatis* DNA fragments (clinical sample, the species identification was confirmed by direct sequencing of nucleotide sequences).

The analytical specificity was confirmed on the investigating of DNA/RNA of following microorganism/strains:

- strains from ATCC[®] (American Type Culture Collection, USA): *Neisseria gonorrhoeae* ATCC[®] 49926TM, *Gardnerella vaginalis* ATCC[®] 14018TM, *Streptococcus agalactiae* ATCC[®] 13813TM, *Streptococcus pyogenes* ATCC[®] 19615TM in concentration no less than 1x10⁵ copies/ml;
- clinical samples (the species identification was confirmed by direct sequencing of nucleotide sequences): *Atopobium vaginae*, *Candida albicans*, *Candida glabrata*, *Chlamydia trachomatis*, *Cytomegalovirus humanbeta5*, *Lymphocryptovirus humangamma4*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Simplexvirus humanalpha1*, *Simplexvirus humanalpha2*, *Trichomonas vaginalis*, *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Varicellovirus humanalpha3* in concentration no less than 1x10⁵ GE/ml;
- clinical samples (the species identification was confirmed by direct sequencing of nucleotide sequences): *Human papillomavirus* belonging to types of low, possibly high carcinogenic risk (possibly HCR) and uncertain risk, in particular types 6, 11, 42, 44, 44, 62, 67, 70, 72, 72, 73, 81, 82, 84 at a concentration of at least 1x10⁵ copies/ml;
- human DNA in concentration of 1 mg/ml.

Nonspecific responses as well as cross-reactions between HPV types when using highly concentrated samples were absent. The specificity of the test was confirmed by direct sequencing of nucleotide sequences.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Reproducibility, repeatability and trueness

Repeatability and reproducibility were determined by testing of positive model samples. Positive model samples were dilutions of standard control samples containing HPV HCR DNA of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 types to final concentrations of 1x10⁴ and 1x10⁷ copies/ml in samples of urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix)), in which HPV DNA of types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 was not previously detected (see Tables 3, 4).

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing in two independent laboratories, by different operators, on different days, on different instruments, different PCR reagent kit series.

Table 10

Repeatability

DNA extraction kit	HPV type	Expected concentration on value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Standard deviation (SD)	Coefficient of variation (CV), %
DNA-sorb-AM	HPV type 16	7.00	10	7.13	0.11	1.56
		4.00	10	4.06	0.16	4.00
	HPV type 18	7.00	10	7.09	0.11	1.50
		4.00	10	3.99	0.02	0.44
	HPV type 31	7.00	10	7.11	0.10	1.44
		4.00	10	4.02	0.03	0.66
	HPV type 33	7.00	10	7.11	0.12	1.73
		4.00	10	4.00	0.03	0.66
	HPV type 35	7.00	10	7.12	0.09	1.30
		4.00	10	4.00	0.03	0.73
	HPV type 39	7.00	10	7.14	0.09	1.28
		4.00	10	4.17	0.06	1.50
	HPV type 45	7.00	10	7.08	0.10	1.38
		4.00	10	4.17	0.08	1.95
	HPV type 51	7.00	10	7.13	0.09	1.33
		4.00	10	4.16	0.06	1.51
	HPV type 52	7.00	10	7.12	0.11	1.51
		4.00	10	4.16	0.07	1.74
	HPV type 56	7.00	10	7.14	0.08	1.17
		4.00	10	4.00	0.03	0.69
	HPV type 58	7.00	10	7.08	0.12	1.68
		4.00	10	4.20	0.07	1.75
	HPV type 59	7.00	10	7.16	0.10	1.37
		4.00	10	4.01	0.03	0.69
	HPV type 66	7.00	10	7.20	0.06	0.84
		4.00	10	4.11	0.07	1.72
	HPV type 68	7.00	10	7.15	0.13	1.75
		4.00	10	4.16	0.08	1.93

⁵ Number of copies of virus DNA in the biological material placed in the specified transport medium and calculated per 1 ml.

DNA extraction kit	HPV type	Expected concentration on value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Standard deviation (SD)	Coefficient of variation (CV), %
AmpliSens [®] MAGNO-sorb-URO	HPV type 16	7.00	10	7.14	0.12	1.67
		4.00	10	4.06	0.13	3.24
	HPV type 18	7.00	10	7.08	0.11	1.51
		4.00	10	4.01	0.02	0.58
	HPV type 31	7.00	10	7.12	0.12	1.69
		4.00	10	4.01	0.02	0.43
	HPV type 33	7.00	10	7.12	0.07	1.01
		4.00	10	4.00	0.03	0.64
	HPV type 35	7.00	10	7.15	0.09	1.21
		4.00	10	3.99	0.03	0.71
	HPV type 39	7.00	10	7.12	0.11	1.49
		4.00	10	4.16	0.05	1.16
	HPV type 45	7.00	10	7.16	0.10	1.34
		4.00	10	4.16	0.06	1.37
	HPV type 51	7.00	10	7.14	0.08	1.08
		4.00	10	4.19	0.07	1.62
	HPV type 52	7.00	10	7.11	0.13	1.81
		4.00	10	4.19	0.06	1.52
	HPV type 56	7.00	10	7.07	0.08	1.15
		4.00	10	4.00	0.02	0.54
	HPV type 58	7.00	10	7.14	0.11	1.60
		4.00	10	4.16	0.05	1.31
	HPV type 59	7.00	10	7.19	0.07	1.03
		4.00	10	4.02	0.02	0.41
	HPV type 66	7.00	10	7.17	0.10	1.41
		4.00	10	4.18	0.05	1.25
	HPV type 68	7.00	10	7.15	0.07	0.97
		4.00	10	4.19	0.07	1.60
AmpliSens [®] DNA-sorb-D	HPV type 16	7.00	10	7.10	0.10	1.40
		4.00	10	4.08	0.15	3.73
	HPV type 18	7.00	10	7.19	0.08	1.17
		4.00	10	4.01	0.03	0.65
	HPV type 31	7.00	10	7.07	0.12	1.70
		4.00	10	4.01	0.03	0.64
	HPV type 33	7.00	10	7.06	0.08	1.10
		4.00	10	3.99	0.02	0.58
	HPV type 35	7.00	10	7.12	0.11	1.51
		4.00	10	4.01	0.02	0.45
	HPV type 39	7.00	10	7.13	0.12	1.64
		4.00	10	4.15	0.06	1.45
	HPV type 45	7.00	10	7.14	0.10	1.40
		4.00	10	4.15	0.10	2.39
	HPV type 51	7.00	10	7.07	0.12	1.75
		4.00	10	4.18	0.08	1.96
	HPV type 52	7.00	10	7.10	0.13	1.83
		4.00	10	4.18	0.08	1.94
	HPV type 56	7.00	10	7.15	0.10	1.39
		4.00	10	3.99	0.02	0.53
	HPV type 58	7.00	10	7.08	0.12	1.67
		4.00	10	4.16	0.08	1.82
	HPV type 59	7.00	10	7.13	0.09	1.30
		4.00	10	4.01	0.02	0.57
	HPV type 66	7.00	10	7.13	0.11	1.55
		4.00	10	4.17	0.07	1.74
	HPV type 68	7.00	10	7.13	0.13	1.80
		4.00	10	4.18	0.07	1.71

Table 11

Reproducibility

DNA extraction kit	HPV type	Expected concentration on value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Standard deviation (SD)	Coefficient of variation (CV), %
DNA-sorb-AM	HPV type 16	7.00	40	7.11	0.21	2.99
		4.00	40	4.02	0.16	3.90
	HPV type 18	7.00	40	7.09	0.21	2.89
		4.00	40	4.00	0.05	1.23
	HPV type 31	7.00	40	7.10	0.20	2.81
		4.00	40	4.01	0.05	1.21
	HPV type 33	7.00	40	7.13	0.22	3.15
		4.00	40	4.01	0.05	1.19
	HPV type 35	7.00	40	7.12	0.20	2.78
		4.00	40	4.00	0.06	1.38
	HPV type 39	7.00	40	7.08	0.21	3.00
		4.00	40	4.16	0.12	2.89
	HPV type 45	7.00	40	7.13	0.16	2.27
		4.00	40	4.17	0.15	3.52
	HPV type 51	7.00	40	7.14	0.21	3.01
		4.00	40	4.17	0.14	3.43
	HPV type 52	7.00	40	7.14	0.21	2.87
		4.00	40	4.18	0.12	2.96
	HPV type 56	7.00	40	7.12	0.21	2.99
		4.00	40	4.04	0.12	2.88
	HPV type 58	7.00	40	7.10	0.21	2.92
		4.00	40	4.09	0.17	4.16
	HPV type 59	7.00	40	7.11	0.20	2.80
		4.00	40	4.00	0.06	1.41
	HPV type 66	7.00	40	7.16	0.20	2.77
		4.00	40	4.15	0.15	3.54
	HPV type 68	7.00	40	7.13	0.21	2.99
		4.00	40	4.17	0.15	3.53

DNA extraction kit	HPV type	Expected concentration on value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Standard deviation (SD)	Coefficient of variation (CV), %
AmpliSens® MAGNO-sorb-URO	HPV type 16	7.00	40	7.12	0.22	3.15
		4.00	40	4.02	0.13	3.27
	HPV type 18	7.00	40	7.12	0.22	3.04
		4.00	40	4.01	0.05	1.25
	HPV type 31	7.00	40	7.12	0.23	3.18
		4.00	40	4.01	0.04	0.95
	HPV type 33	7.00	40	7.13	0.20	2.77
		4.00	40	4.00	0.05	1.30
	HPV type 35	7.00	40	7.14	0.20	2.75
		4.00	40	4.00	0.05	1.26
	HPV type 39	7.00	40	7.15	0.19	2.69
		4.00	40	4.17	0.12	2.90
	HPV type 45	7.00	40	7.12	0.22	3.11
		4.00	40	4.00	0.04	1.12
	HPV type 51	7.00	40	7.12	0.19	2.63
		4.00	40	4.17	0.14	3.39
	HPV type 52	7.00	40	7.12	0.24	3.38
		4.00	40	4.17	0.12	2.95
	HPV type 56	7.00	40	7.11	0.22	3.07
		4.00	40	4.04	0.13	3.16
	HPV type 58	7.00	40	7.12	0.21	2.91
		4.00	40	4.10	0.16	4.00
	HPV type 59	7.00	40	7.13	0.22	3.04
		4.00	40	4.00	0.05	1.23
	HPV type 66	7.00	40	7.14	0.21	2.95
		4.00	40	4.15	0.12	2.98
	HPV type 68	7.00	40	7.12	0.21	3.00
		4.00	40	4.18	0.14	3.24
AmpliSens® DNA-sorb-D	HPV type 16	7.00	40	7.11	0.20	2.85
		4.00	40	4.02	0.15	3.80
	HPV type 18	7.00	40	7.09	0.20	2.77
		4.00	40	4.00	0.05	1.33
	HPV type 31	7.00	40	7.13	0.20	2.83
		4.00	40	4.01	0.05	1.17
	HPV type 33	7.00	40	7.11	0.20	2.75
		4.00	40	4.00	0.05	1.34
	HPV type 35	7.00	40	7.11	0.23	3.27
		4.00	40	4.00	0.05	1.19
	HPV type 39	7.00	40	7.03	0.22	3.08
		4.00	40	4.16	0.13	3.12
	HPV type 45	7.00	40	7.13	0.19	2.73
		4.00	40	4.00	0.05	1.16
	HPV type 51	7.00	40	7.10	0.22	3.12
		4.00	40	4.18	0.13	3.20
	HPV type 52	7.00	40	7.12	0.21	2.92
		4.00	40	4.17	0.13	3.21
	HPV type 56	7.00	40	7.15	0.21	2.96
		4.00	40	4.05	0.16	3.85
	HPV type 58	7.00	40	7.11	0.21	2.90
		4.00	40	4.08	0.16	3.82
	HPV type 59	7.00	40	7.13	0.19	2.72
		4.00	40	4.00	0.05	1.28
	HPV type 66	7.00	40	7.12	0.22	3.09
		4.00	40	4.17	0.13	3.07
	HPV type 68	7.00	40	7.12	0.22	3.03
		4.00	40	4.19	0.13	3.20

The trueness was determined by testing positive model samples. Positive model samples were dilutions of standard control samples containing HPV/HCV DNA 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 types up to a final concentration of 1x10⁴ and 1x10⁷ copies/ml in samples of urogenital mucous discharge (vaginal swab, epithelial scrapes from the cervical mucous membrane (ectocervix and endocervix) in which preliminary HPV DNA of the declared types was not detected. (See Table 12).

Table 12

Trueness

DNA extraction kit	HPV type	Expected concentration value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Bias (B), %
DNA-sorb-AM	HPV type 16	7.00	30	7.11	1.57
		4.00	30	4.02	0.62
	HPV type 18	7.00	30	7.09	1.24
		4.00	30	4.00	-0.02
	HPV type 31	7.00	30	7.10	1.50
		4.00	30	4.01	0.30
	HPV type 33	7.00	30	7.13	1.81
		4.00	30	4.01	0.22
	HPV type 35	7.00	30	7.13	1.80
		4.00	30	4.00	0.12
	HPV type 39	7.00	30	7.12	1.74
		4.00	30	4.17	4.19
	HPV type 45	7.00	30	7.12	1.70
		4.00	30	4.17	4.24
	HPV type 51	7.00	30	7.14	2.04
		4.00	30	4.17	4.23
	HPV type 52	7.00	30	7.13	1.85
		4.00	30	4.18	4.58
	HPV type 56	7.00	30	7.12	1.76
		4.00	30	4.00	0.00
	HPV type 58	7.00	30	7.10	1.43
		4.00	30	4.13	3.16
	HPV type 59	7.00	30	7.11	1.57
		4.00	30	4.00	0.06
	HPV type 66	7.00	30	7.12	1.70
		4.00	30	4.16	4.06
	HPV type 68	7.00	30	7.12	1.70
		4.00	30	4.19	4.76

DNA extraction kit	HPV type	Expected concentration value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Bias (B), %
AmpliSens® MAGNO-sorb-URO	66	4.00	30	4.14	3.42
		7.00	30	7.11	1.59
	HPV type 68	4.00	30	4.17	4.30
		7.00	30	7.12	1.75
	HPV type 16	4.00	30	4.02	0.58
		7.00	30	7.12	1.68
	HPV type 18	4.00	30	4.01	0.21
		7.00	30	7.11	1.60
	HPV type 31	4.00	30	4.01	0.37
		7.00	30	7.13	1.90
	HPV type 33	4.00	30	4.00	-0.04
		7.00	30	7.14	1.94
	HPV type 35	4.00	30	4.00	-0.06
		7.00	30	7.11	1.63
	HPV type 39	4.00	30	4.17	4.25
		7.00	30	7.12	1.69
	HPV type 45	4.00	30	4.17	4.29
		7.00	30	7.12	1.68
	HPV type 51	4.00	30	4.17	4.35
		7.00	30	7.13	1.83
	HPV type 52	4.00	30	4.16	4.04
		7.00	30	7.11	1.59
	HPV type 56	4.00	30	4.00	0.08
		7.00	30	7.13	1.80
	HPV type 58	4.00	30	4.12	3.06
		7.00	30	7.13	1.91
	HPV type 59	4.00	30	4.00	0.01
		7.00	30	7.15	2.13
HPV type 66	4.00	30	4.16	4.08	
	7.00	30	7.14	1.93	
HPV type 68	4.00	30	4.19	4.64	
	7.00	30	7.11	1.51	
AmpliSens® DNA-sorb-D	HPV type 16	4.00	30	4.03	0.73
		7.00	30	7.09	1.26
	HPV type 18	4.00	30	3.99	-0.18
		7.00	30	7.11	1.53
	HPV type 31	4.00	30	4.01	0.30
		7.00	30	7.11	1.51
	HPV type 33	4.00	30	3.99	-0.14
		7.00	30	7.12	1.69
	HPV type 35	4.00	30	4.00	0.08
		7.00	30	7.11	1.55
	HPV type 39	4.00	30	4.15	3.87
		7.00	30	7.13	1.86
	HPV type 45	4.00	30	4.16	4.07
		7.00	30	7.11	1.53
	HPV type 51	4.00	30	4.19	4.71
		7.00	30	7.13	1.84
	HPV type 52	4.00	30	4.18	4.43
		7.00	30	7.13	1.88
	HPV type 56	4.00	30	4.00	-0.12
		7.00	30	7.11	1.62
	HPV type 58	4.00	30	4.11	2.63
		7.00	30	7.13	1.80
	HPV type 59	4.00	30	4.00	0.07
		7.00	30	7.11	1.61
	HPV type 66	4.00	30	4.16	4.06
		7.00	30	7.12	1.70
	HPV type 68	4.00	30	4.19	4.76

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15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Total Quality Management System, each lot of **AmpliSens® HPV HCR screen-titre-14-FRT** PCR kit is tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
04.10.24 HM	Through the text	The text formatting was changed
	1. Intended use	The intended use was specified. The list of biological material was expanded. The subsection <i>Indications and contra-indications for use of the reagent kit</i> was added
	2. Principle of PCR detection	The section was rewritten
	3. Content	PCR-mix-FL HPV 14 reagent description was changed
	4. Additional requirements	The section was actualized and updated with materials and instruments, Rotor-Gene 6000 was added
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection <i>Interfering substances and limitations of using test material samples</i> was added
	7. Working conditions	Relative humidity range was added
	8. Protocol	Working procedure was rewritten
	9. Data Analysis	Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables
	10. Troubleshooting	The section was rewritten
	13. Specifications	The list of microorganisms/strains to prove the analytical specificity was expanded. The subsection <i>13.3. Reproducibility, repeatability and trueness</i> was added
	14. References	References were renewed

