AmpliSens® *HPV* 16/18-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
	Caution		

1. INTENDED USE

AmpliSens® *HPV* 16/18-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection and differentiation of genotypes 16 and 18 of *Human Papillomavirus* (*HPV*) DNA in the clinical material (urogenital swabs) using real-time hybridization-fluorescence detection of amplified products.

AmpliSens® *HPV* 16/18-FRT PCR kit, which allows differentiation of two most carcinogenic virus genotypes, is recommended as an auxiliary tool for detection of *papillomavirus* infection. PCR kits that allow diagnostics of a wide range (11–14 genotypes) of highly carcinogenic *HPV* genotypes, such as AmpliSens® *HPV* HCR screen-EPh (electrophoretic detection in agarose gel), AmpliSens® *HPV* HCR screen-FRT 2x, AmpliSens® *HPV* HCR screen-FRT 3x, and AmpliSens® *HPV* HCR screen-titre-FRT (hybridization fluorescence detection) should be used at the first stage of diagnosing *HPV.*

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

HPV genotypes 16 and 18 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *HPV* 16/18 primers. In 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 the fluorescence intensities during the real-time PCR allows the detection of the accumulating product without re-opening the reaction tubes after the PCR run.

Principle of testing is based on simultaneous amplification of *HPV* DNA fragments and a fragment of the β-globin gene, which is used as an endogenous internal control. PCR analysis for the presence of *HPV* genotypes 16 and 18 DNA is performed in the same tube.

The DNA target used as an endogenous internal control is the fragment of human genome and should be present in a sample (cervical swab) in a sufficient amount equivalent to the amount of cells in the sample (10^5–10^7 cells/ml). Therefore, an endogenous internal control makes it possible not only to monitor the stages of the test (DNA extraction and PCR) but also to assess the adequacy of clinical material collection and storage. If the amount of epithelial cells in the specimen is insufficient, the amplification signal of the β-globin gene will be low.

AmpliSens® *HPV* 16/18-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT, "hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C. In variant FRT-100 F, "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.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA-target	*HPV* genotype 16 DNA	*HPV* genotype 18 DNA	IC DNA
Target gene	*gene* E7	*gene* E7	DNA fragment of β-globin gene

3. CONTENT

AmpliSens® *HPV* 16/18-FRT PCR kit is produced in 2 forms:
variant FRT-100 F, REF R-V12-F-CE
variant FRT REF R-V12-100-CE.

Variant FRT-100 F includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FL *HPV* 16/18		clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-mix-2-FRT		colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)		colorless clear liquid	0.03	2 tubes
DNA calibrator	**C1 *HPV* 16, 18**	colorless clear liquid	0.04	3 tubes
	C2 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
	C3 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
DNA-buffer		colorless clear liquid	0.5	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as the Negative Control of Extraction.
Variant FRT-100 F is intended for 110 reactions (including controls).

Variant FRT includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FL *HPV* 16/18 ready-to-use single-dose test tubes (*under wax)*		clear liquid from colorless to light lilac colour	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red		red clear liquid	1.1	1 tube
DNA calibrator	**C1 *HPV* 16, 18**	colorless clear liquid	0.04	3 tubes
	C2 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
	C3 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
DNA-buffer		colorless clear liquid	0.5	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as the Negative Control of Extraction.
Variant FRT is intended for 110 reactions (including controls).

Variant FRT and **Variant FRT-100 F** include the software in Microsoft Excel format for processing of data and generation of results.

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ or iCycler iQ5 (Bio-Rad, USA), Mx3000P or Mx3005P (Stratagene, USA)).
- Disposable polypropylene tubes for PCR kit variant FRT-100 F.
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 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.
- Refrigerator for 2–8 °C.
- Deep-freezer for the temperature from minus 24 to minus 16 °C.
- 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 (specimens, 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 afterwards.
- 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance 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 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 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 in 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

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® *HPV* 16/18-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (urogenital swabs).

Female: samples of epithelial cells should be obtained as for cytological examination:

Method 1. The sampling kit with one/two cervical cytobrushes and 2-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 953-CE are used.

Place the cervical epithelial scrape (endocervix) taken with the first cervical cytobrush and/or the superficial cervical scrape (ectocervix) taken with the second cervical cytobrush to the tube with transport medium. Break the lower part of the cytobrush and leave it in the tube with transport medium.

Method 2. The Digene cervical sampler (USA), which contains cervical cytobrush and a tube with 1.0 ml of Digene transport medium, is used.
Place the cervical epithelial scrape (endocervix) obtained with cytobrush into the tube with Digene transport medium.

Method 3. The sampling kit, which contains the combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix and 5-ml tube with 2.0 ml of **Transport Medium with Mucolytic Agent** REF 952-CE, is used.

Place the endocervix and ectocervix into the tube with transport medium. Break the lower part of the probe and leave it in the tube with transport medium.

Method 4. The sampling kit, which contains the combined gynecological probe for simultaneously taking epithelial samples from endocervix and ectocervix and a liquid-based cytology vial with CytoScreen (Italy) or PreservCyt (USA) transport medium, is used.
Place the endocervix and ectocervix into the tube with transport medium. Break the lower part of the probe and leave it in the vial with transport medium.

Male: Obtain urethral epithelial scrape by universal probe, place it into the 2-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 953-CE.

Storage conditions:

- at the temperature from 18 to 25 °C – no more than 5 days;
- at the temperature from 2 to 8 °C – no more than 20 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year. Only one freeze-thawing cycle is allowed;
- in the transport medium for liquid-based cytology at room temperature – for 1 year.

7. WORKING CONDITIONS

AmpliSens® *HPV* 16/18-FRT PCR kit should be used at 18–25 °C

8. PROTOCOL

8.1. DNA Extraction

It is recommended to use the following nucleic acid extraction kit:

— **DNA-sorb-AM**, REF K1-12-100-CE.

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– — Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

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

NOTE: It is possible to transfer the whole volume of homogenized Universal Sorbent to the tube with Lysis Solution (2 ml of Universal Sorbent per 30 ml of Lysis Solution). Prepared mixture can be stored at room temperature for up to 2 days. Stir thoroughly before use. Transfer 320 µl of prepared mixture to each tube.

8.2. Preparing PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

8.2.1 Preparing tubes for PCR

Variant FRT

Total reaction volume is **30 µl**, the volume of the DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FL *HPV* 16/18** for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer of each tube so that it does not fall under the wax and mix with **PCR-mix-1-FL *HPV* 16/18.**
3. Add **10 µl** of **DNA samples** obtained at DNA extraction stage.
4. Carry out the control amplification reactions:

For qualitative analysis:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **DNA calibrator C2 *HPV* 16, 18** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl of sample extracted from Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

For quantitative analysis:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C– - Add **10 µl of sample extracted from Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

DNA calibrators:

C1 - Add **10 µl** of **DNA calibrator C1 *HPV* 16, 18,** to the tube labeled C1.

C2 - Add **10 µl** of **DNA calibrator C2 *HPV* 16, 18,** to the tube labeled C2.

C3 - Add **10 µl** of **DNA calibrator C3 *HPV* 16, 18,** to the tube labeled C3

Variant FRT-100 F

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).** To do this, transfer the whole content of the tube with **polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)**. Vortex carefully to avoid foaming. Indicate the date of mixture preparation on the tube.

NOTE: The prepared mixture is intended for 60 samples. The mixture can be stored at 2–8 °C for up to 3 month and used as required.

2. Prepare the reaction mixture (Table 2 and 3). When calculating the volume of the mixture, take into account the necessity to run **three control reactions for qualitative analysis** and **five control reactions for quantitative analysis**. Do not forget to add extra volumes for one more reaction.
   Each PCR reaction requires:
   - **10 µl** of **PCR-mix-1-FL *HPV* 16/18;**
   - **5 µl** of the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).**

Table 2

Scheme of reaction mixture preparation for qualitative analysis

Number of clinical samples	4	5	6	7	8	9	10	11	12	13	14	15	16	17
PCR-mix-1-FL *HPV* 16/18, µl	80	90	100	110	120	130	140	150	160	170	180	190	200	210
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	40	45	50	55	60	65	70	75	80	85	90	95	100	105
Number of clinical samples	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
PCR-mix-1-FL *HPV* 16/18, µl	220	230	240	250	260	270	280	290	300	310	320	330	340	350
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	110	115	120	125	130	135	140	145	150	155	160	165	170	175

The calculation scheme for samples is given according to formula **n + 4,** where:
n is the number of clinical samples for analysis**;**
4 is the number of controls of PCR analysis (**1** Control of Extraction, **2** Controls of amplification, and **1** extra tube).

Table 3

Scheme of reaction mixture preparation for quantitative analysis

Number of clinical samples	4	5	6	7	8	9	10	11	12	13	14	15	16	17
PCR-mix-1-FL *HPV* 16/18, µl	100	110	120	130	140	150	160	170	180	190	200	210	220	230
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	50	55	60	65	70	75	80	85	90	95	100	105	110	115
Number of clinical samples	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
PCR-mix-1-FL *HPV* 16/18, µl	240	250	260	270	280	290	300	310	320	330	340	350	360	370
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	120	125	130	135	140	145	150	155	160	165	170	175	180	185

The calculation scheme for samples is given according to formula **n + 6,** where:
n is the number of clinical samples for analysis**;**
6 is the number of controls of PCR analysis (**1** Control of Extraction, **4** Controls of amplification, and **1** extra tube).

3. Prepare the required number of tubes for amplification of DNA from clinical and control samples. Transfer **15** µl of the prepared mixture to each tube.

4. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes.
5. Carry out the control amplification reactions:

For qualitative analysis:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **DNA calibrator C2 *HPV* 16, 18** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl of sample extracted from Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

For quantitative analysis:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C– - Add **10 µl of sample extracted from Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

DNA calibrators:

C1 - Add **10 µl** of **DNA calibrator C1 *HPV* 16, 18,** to the tube labeled C1.

C2 - Add **10 µl** of **DNA calibrator C2 *HPV* 16, 18,** to the tube labeled C2.

C3 - Add **10 µl** of **DNA calibrator C3 *HPV* 16, 18,** to the tube labeled C3

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 4a

AmpliSens-1 amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**5 s**	**5**	**95**	**5 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**5 s**	**40**	**95**	**5 s**	**40**
	60	**20 s *fluorescent signal detection***		**60**	**30 s *fluorescent signal detection***	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the FAM, JOE, and ROX fluorophores (other channels are enabled if several tests are simultaneously performed in a single run).
DNA *HPV* 16-18 amplification program can be applied as well (see Table 4b)

Table 4b

DNA HPV 16-18 amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	15 s	45	**95**	20 s	45
	60	35 s *fluorescent signal detection*		**60**	1 min *fluorescent signal detection*	

Fluorescent signal is detected in the channels for the FAM, JOE, and ROX fluorophores.
2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument..

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels:
— ***HPV* genotype 16 DNA** is detected in the channel for the FAM fluorophore (or analogous depending on the instrument);
— ***HPV* genotype 18 DNA** is detected in the channel for the JOE fluorophore (or analogous depending on the instrument);
— Endogenous internal control (human DNA fragment) is detected in the channel for the ROX fluorophore (or analogous depending on the instrument).
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.
Principle of interpretation is following:

For qualitative analysis (see Table 5):
— ***HPV* genotype 16 DNA** is **detected** in the sample if its *Ct* value determined in the results grid in the channel for the FAM fluorophore does not exceed the specified boundary *Ct* value.
— ***HPV* genotype 18 DNA** is **detected** in the sample if its *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the specified boundary *Ct* value.
— ***HPV* genotypes 16 and 18 DNA** are **not detected** in the sample if their *Ct* values determined in the results grid in the channels for the FAM and JOE fluorophores are greater than the specified boundary *Ct* values or are not detected, whereas the *Ct* value determined in the results grid in channel for the ROX fluorophore does not exceed the specified boundary *Ct* value.
— The result is **invalid** if the *Ct* value detected in the result grid in the channel for the ROX fluorophore is greater than the specified boundary *Ct* value or is not detected.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

Table 5

General interpretation of results

FAM (*HPV* 16)	JOE (*HPV* 18)	ROX (IC)	Result
–	–	+	***HPV* genotypes 16 and 18 are not detected**
+	–	+	***HPV* genotype 16 is detected**
+	–	–	
–	+	+	***HPV* genotype 18 is detected**
–	+	–	
+	+	+	***HPV* genotypes 16 and 18 are detected**
+	+	–	
–	–	–	**Invalid result**

For quantitative analysis:
Based on the obtained *Ct* values and specified concentration values of DNA calibrators a calibration line is plotted and the number of copies of *HPV* genotype 16 DNA, *HPV* genotype 18 DNA as well as human DNA per PCR sample is calculated. The obtained data are used to calculate the amount of *HPV* genotype 16 or/and 18 DNA per 100,000 human cells.

$$\lg\left[\frac{\text{quantity of } HPV \text{ (16 or 18) DNA copies}}{\text{quantity of human DNA copies}} \times 200{,}000\right] = \lg(HPV \text{ per } 100{,}000 \text{ cells})$$

The obtained result is interpreted according to the table below.

Table 6

Lg(*HPV* per 100,000 cells) data interpretation

Lg (*HPV* per 100,000 cells)	**Interpretation**
<3	Clinically insignificant
3–5	Clinically significant. Dysplasia cannot be excluded. Risk of dysplasia development
>5	Clinically significant, increased. Dysplasia is highly expectable

NOTE: The DNA calibrator concentrations are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The results of analysis (for both qualitative and quantitative assays) are considered reliable only if the results obtained for DNA calibrators, Negative Control of amplification, and Negative Controls of extraction are correct (see Table 7).

Table 7

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**		
		FAM	**JOE**	**ROX**
C–	DNA extraction	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent
C1 C2 C3	PCR	< boundary value	< boundary value	< boundary value

10. TROUBLESHOOTING

Results of <u>quantitative analysis</u> are not taken into account in the following cases:
1. If the human DNA concentration is less than 10^3 GE per reaction (the value determined for the samples in the channel for the ROX fluorophore), this means that either an insufficient amount of clinical sample was taken or that errors had occurred during the sample treatment. The sample should be analyzed once again starting from the DNA extraction stage.
2. If the correlation coefficient R^2 for calibration curve is less than 0.9, all samples should be re-examined starting from the DNA extraction stage.

Results of both <u>qualitative and quantitative analysis</u> are not taken into account in the following cases:
1. If a *Ct* value appears for the Negative Control of amplification (NCA) and/or for the Negative Control of extraction (C–) in the channels for the FAM, JOE, and/or ROX fluorophores, this indicates contamination of samples or reagents. It is necessary to repeat the analysis of all samples in which *HPV* DNA was detected and to eliminate the source of contamination.
2. If *Ct* values of DNA calibrators (C1 *HPV* 16, 18, C2 *HPV* 16, 18, and C3 *HPV* 16, 18) in the channels for the FAM, JOE, and/or ROX fluorophores are absent or greater than the specified boundary values, PCR should be repeated for all samples in which *HPV* DNA was not detected.
3. If the *Ct* value of a sample is not detected or exceeds the boundary *Ct* value specified for channels for the FAM, and/or JOE fluorophores, whereas the *Ct* value detected in the channel for the ROX fluorophore exceeds the specified boundary *Ct* value, the analysis should be repeated starting from the DNA extraction stage. This may be caused by the presence of PCR inhibitors or by loss of DNA during clinical sample handling.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *HPV* 16/18-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* 16/18-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FL *HPV* 16/18, PCR-mix-2-FRT and polymerase (TaqF) included in the PCR kit variant FRT-100 F). All components of the **AmpliSens® *HPV* 16/18-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FL *HPV* 16/18, PCR-mix-2-FRT, and polymerase (TaqF) (included in the PCR kit variant FRT-100 F) are to be stored at the temperature from minus 24 to minus 16 ºC

NOTE: PCR-mix-1-FL *HPV* 16/18 is to be kept away from light

[1] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).
[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P, Mx3005P (Stratagene, USA)

13. SPECIFICATIONS

13.1. Sensitivity

Table 8

Clinical material	Transport medium	DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml [3]
Cervical swab	Transport Medium with Mucolytic Agent	**DNA-sorb-AM**	variants FRT, FRT-100 F	$1x10^3$

Linear range of the PCR kit is from 10^3 to 10^8 GE/ml for each target.

13.2. Specificity

The analytical specificity of **AmpliSens® *HPV* 16/18-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.

AmpliSens® *HPV* 16/18-FRT PCR kit detects a fragment of DNA of *HPV* genotypes 16 and 18. The analytical specificity of the PCR kit was investigated by adding to the reaction DNA/RNA of different microorganisms (*adenovirus* types 2, 3 and 7, *cytomegalovirus, Epstein-Barr virus*, *varicella zoster virus*, *hepatitis* B and C, *human immunodeficiency virus* type 1, *human herpes virus t*ype 6 and 8, *herpes simplex virus*, *Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Neisseria gonorrhoeae, Trichomonas vaginalis, Candida albicans, Streptococcus pyogenes, Staphylococcus aureus*, the DNA of human papillomavirus types β, γ, μ (1, 3, 4, 5, 8, 37, 38, 65, 20, 24, 49, 50, 15), type α of low and unknown carcinogenicity risk (6, 11, 26, 53, 7, 27, 10) and type α of high carcinogenicity risk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 67, 68) at a concentration of 10^8 copies of *HPV* DNA per ml). Cross-reactivity was not observed.

The clinical specificity of **AmpliSens® *HPV* 16/18-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal State Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to **AmpliSens® *HPV* 16/18-FRT** PCR kit for qualitative and quantitative detection and differentiation of genotypes 16 and 18 of *human papillomaviruses* (*HPV*) DNA in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *HPV* 16/18-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.10.12 LA	Protocol 8.2.3. Amplification	“DNA HPV 16-18” amplification program was added (table 3b)
18.06.14 PM	1. Intended use	PCR kit intended use was specified (“qualitative detection” was changed to “qualitative and quantitative detection”).
	8. Protocol	Information about extraction methods was added.
	Through the text	Instruction manual was corrected in accordance with the template
03.02.16 ME	Text	Corrections according to the template, grammar corrections
	8.1. DNA extraction	Information about control of extraction was added
12.11.18 EM	3. Content	The colour of the reagent was specified
11.10.19 PM	Through the text	Corrections according to the template. The text formatting was changed
	2. Principle of PCR-detection	The table with target genes was added
	5. General precautions	The chapter was updated
	Footer, 3. Content	REF R-V12-Mod(RG,iQ,Mx)-CE was changed to REF R-V12-F-CE REF R-V12-100(iQ,Mx,Dt)-CE, REF R-V12-100(RG)-CE were changed to REF R-V12-100-CE
03.06.20 VA	Footer	The phrase “Not for use in the Russian Federation” was added
24.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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[3] Number of genome equivalents of microorganism (GE) per 1 ml of clinical sample placed in the specified transport medium