AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection and differentiation of DNA of *human papillomaviruses* of high carcinogenic risk (*HPV* HCR) in the biological material (urogenital swabs, biopsy material of cervical mucous membrane) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

HPV HCR detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. 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.

The method is based on simultaneous real-time amplification (multiplex PCR) of DNA fragments of *HPV* genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and a DNA fragment of β-globin gene in one tube. DNA fragment of β-globin gene is used as an internal endogenous control. PCR analysis for the presence of DNA of 14 *HPV* types is carried out in four tubes. Each genotype is detected in separate fluorescent channel that makes it possible not only to detect but also to determine the genotype and concentration of detected *human papillomaviruses* of high carcinogenic risk. The DNA target selected as an endogenous internal control is a human genome fragment. It must be always present in the sample (cervical swab) in sufficient quantities equivalent to the number of cells in the swab (10^3–10^5 genome equivalents). Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and PCR amplification) but also to assess the adequacy of sampling and storage of clinical material. If epithelial swab was taken incorrectly (the number of epithelial cells is insufficient), the amplification signal of β-globin gene will be underestimated.

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. The results of amplification of DNA calibrators are used to construct a calibration curve, on the basis of which the concentration of *HPV* DNA in test samples is determined.

AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase 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	Cy5
Name of PCR-mix-FL	**DNA-target**			
HPV 16,18,31 / Glob	DNA of *HPV* HCR genotype 16	DNA of *HPV* HCR genotype 31	DNA of *HPV* HCR genotype 18	DNA fragment of β-globin gene (IC Glob)
HPV 39,45,59 / Glob	DNA of *HPV* HCR genotype 39	DNA of *HPV* HCR genotype 45	DNA of *HPV* HCR genotype 59	DNA fragment of β-globin gene (IC Glob)
HPV 33,35,56,68	DNA of *HPV* HCR genotype 33	DNA of *HPV* HCR genotype 35	DNA of *HPV* HCR genotype 68	DNA of *HPV* HCR genotype 56
HPV 51,52,58,66	DNA of *HPV* HCR genotype 58	DNA of *HPV* HCR genotype 52	DNA of *HPV* HCR genotype 66	DNA of *HPV* HCR genotype 51
Name of PCR-mix-FL	**Target gene**			
HPV 16,18,31 / Glob	E6 gene	E6 gene	E7 gene	β-globin gene
HPV 39,45,59 / Glob	E7 gene	E6 gene	E6 gene	β-globin gene
HPV 33,35,56,68	E6 gene	E6/E7 gene	E6 gene	E1 gene
HPV 51,52,58,66	E6 gene	E7 gene	E6 gene	E7 gene

3. CONTENT

AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit is produced in 1 form: **variant FRT-100 F**, REF R-V67-F-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *HPV* 16,18,31 / Glob	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-mix-FL *HPV* 39,45,59 / Glob	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-mix-FL *HPV* 33,35,56,68	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-mix-FL *HPV* 51,52,58,66	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-buffer-B	colorless clear liquid	0.6	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	4 tubes
Calibrator C1 *HPV* 16,18,31 / Glob	colorless clear liquid	0.2	1 tube
Calibrator C2 *HPV* 16,18,31 / Glob	colorless clear liquid	0.2	1 tube
Calibrator C1 *HPV* 39,45,59 / Glob	colorless clear liquid	0.2	1 tube
Calibrator C2 *HPV* 39,45,59 / Glob	colorless clear liquid	0.2	1 tube
Calibrator C1 *HPV* 33,35,56,68	colorless clear liquid	0.2	1 tube
Calibrator C2 *HPV* 33,35,56,68	colorless clear liquid	0.2	1 tube
Calibrator C1 *HPV* 51,52,58,66	colorless clear liquid	0.2	1 tube
Calibrator C2 *HPV* 51,52,58,66	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see **DNA-sorb-AM** REF K1-12-100-CE, **DNA-sorb-C** REF K1-6-50-CE, **AmpliSens® DNA-sorb-D** REF K8-2331-100-CE protocols).

AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit is intended for 440 amplification reactions (110 tests), including controls.

Compact disk with the software in Microsoft Excel format for automatic processing of data and generation of results is enclosed in PCR kit.

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ5 (Bio-Rad, USA); Mx3000P, Mx3005P (Stratagene, USA), CFX96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) or strips of eight 0.2-ml tubes with optical transparent caps:
  a) 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;
  b) strips of four 0.1-ml Rotor-Gene PCR tubes with caps if a rotor-type instrument is used.
- Refrigerator with the range from 2 to 8 °C.
- Deep-freezer with the range 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 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.
- Safety Data Sheets (SDS) are available on request.
- 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

The following clinical material is used for analysis:

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 952-CE; 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) taken with the cervical cytobrush to 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 a 2-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 952-CE; REF 953-CE.

Place the cervical epithelial swab (endocervix) and the superficial cervical swab (ectocervix) into the tube with the 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 cervical epithelial swab (endocervix) and the superficial cervical swab (ectocervix) into the tube with transport medium. Break the lower part of the probe and leave it in the vial with transport medium.

Male: Place the urethral epithelial swab taken with a universal probe to a 2.0-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 952-CE; REF 953-CE.

Sample 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 – no more than 1 year. Only one freeze–thaw cycle of biological material is allowed;
- in the transport medium for liquid-based cytology at room temperature – for 1 year.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR genotype-titre-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 kits:

- **DNA-sorb-AM**, REF K1-12-100-CE for DNA extraction from urogenital swabs obtained by the 1st, 2nd and 3rd method from women and swabs from men;
- **AmpliSens® DNA-sorb-D** REF K8-2331-100-CE for DNA extraction from urogenital swabs obtained by the 4th method (samples for liquid-based cytology);
- **DNA-sorb-C**, REF K1-6-50-CE for DNA extraction from biopsy material of cervical mucous membrane.

In the extraction procedure it is necessary to carry out the control reaction 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.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

For carrying out the study of N biological samples in rotor-type instruments:

a) **in quantitative format** prepare next number of strips (strips of four tubes): **X** (equal to N biological samples) **strips** for test samples + **3 strips** for control samples (8 tubes for calibrators C1, C2 and 4 tubes for DNA sample extracted from Negative Control (C–)). For example, 18 strips are needed for the study of 15 biological samples.

b) **in qualitative format** prepare next number of strips (strips of four tubes): **X** (equal to N biological samples) **strips** for test samples + **2 strips** for control samples (4 tubes for calibrators C2 and 4 tubes for DNA sample extracted from Negative Control (C–)). For example, 17 strips are needed for the study of 15 biological samples.

For carrying out the study of N biological samples in plate-type instruments:

a) **in quantitative format** prepare next number of strips (strips of eight tubes): **X** (equal to 0.5 N biological samples, 0.5 strip is needed for one biological sample, i.e. 4 tubes) **strips** for test samples + **1.5 strip** for control samples (8 tubes for calibrators C1, C2 and 4 tubes for DNA sample extracted from Negative Control (C–)). For example, 12 strips are needed for the study of 21 biological samples.

b) **in qualitative format** prepare next number of strips (strips of eight tubes): **X** (equal to 0.5 N biological samples, 0.5 strip is needed for one biological sample, i.e. 4 tubes) **strips** for test samples + **1 strip** for control samples (4 tubes for calibrators C2 and 4 tubes for DNA sample extracted from Negative Control (C–)). For example, 11.5 strips are needed for the study of 21 biological samples.

Prepare the mixture of **PCR-buffer-B** and **Polymerase (TaqF).** For this, add the whole volume of **polymerase (TaqF) (60 µl)** into the tube with **PCR-buffer-B (600 µl).** Carefully vortex the tube, avoiding foaming. Mark the preparation date on the tube.

NOTE This mix is intended for 30 samples, including controls. Store the prepared mix at the temperature from 2 to 8 °C for 3 months and use as necessary.

For each **PCR-mix-FL *HPV*** mix the reagents for one reaction: **10 µl of PCR-mix-FL *HPV*** and **5 µl of the PCR-buffer-B and Polymerase (TaqF) mixture.** For carrying out the necessary number of reactions including analysis of test and control samples (2 control samples (C2 and C–) for qualitative analysis, 3 control samples (C1, C2 and C–) for quantitative analysis) mix in a new tube the reagents in accordance with the table 2 and 3. Take the reagents with a reserve for one extra reaction.

Table 2

Scheme of reaction mixture preparation for qualitative analysis

Number of test biological samples	Number of test samples including controls	PCR-mix-FL	PCR-buffer-B and Polymerase (TaqF) mixture
3	5	60	30
4	6	70	35
5	7	80	40
6	8	90	45
7	9	100	50
8	10	110	55
9	11	120	60
10	12	130	65
11	13	140	70
12	14	150	75
13	15	160	80
14	16	170	85
15	17	180	90
16[1]	18[1]	190	95
17	19	200	100
18	20	210	105
19	21	220	110
20	22	230	115
21	23	240	120
22[2]	24[2]	250	125

Note – The calculation is given with the formula N+3 for qualitative analysis, where N is the number of test biological samples, 3 includes all needed controls and extra reaction.

Table 3

Scheme of reaction mixture preparation for quantitative analysis

Number of test biological samples	Number of test samples including controls	PCR-mix-FL	PCR-buffer-B and Polymerase (TaqF) mixture
2	5	60	30
3	6	70	35
4	7	80	40
5	8	90	45
6	9	100	50
7	10	110	55
8	11	120	60
9	12	130	65
10	13	140	70
11	14	150	75
12	15	160	80
13	16	170	85
14	17	180	90
15[3]	18[3]	190	95
16	19	200	100
17	20	210	105
18	21	220	110
19	22	230	115
20	23	240	120
21[4]	24[4]	250	125

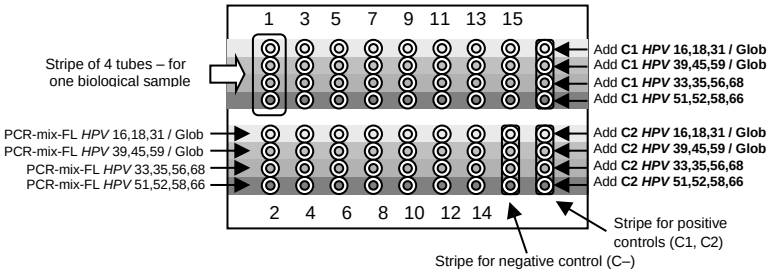
Note – The calculation is given with the formula N+4 for qualitative analysis, where N is the number of test biological samples, 4 includes all needed controls and extra reaction.

NOTE The mix of PCR-mix-FL, PCR-buffer-B and Polymerase (TaqF) is to be used within 2 hour after the preparation.

Add **15 µl** of prepared mixtures in one tube. Due to the fact that analysis of each biological sample is carrying out in 4 tubes with different mixes, it is necessary to obey the following schemes of reagents and biological samples addition:

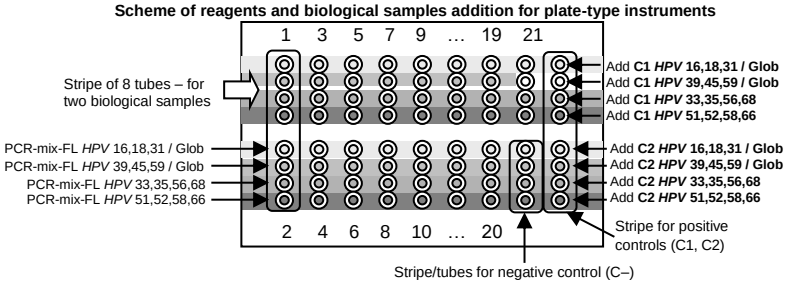
[1] It corresponds to a full load of rotor-type instruments at qualitative analysis.
[2] It corresponds to a full load of plate-type instruments at qualitative analysis.
[3] It corresponds to a full load of rotor-type instruments at quantitative analysis.
[4] It corresponds to a full load of plate-type instruments at quantitative analysis.

Scheme of reagents and biological samples addition for rotor-type instruments



The cells for DNA, obtained in the extraction stage, are numerated

Scheme of reagents and biological samples addition for plate-type instruments



The cells for DNA, obtained in the extraction stage, are numerated.

Add **15 µl** of prepared mix "16,18,31 / Glob" in 1st tube of each strip. Add **15 µl** of prepared mix "39,45,59 / Glob" in 2d tube of each strip. Add **15 µl** of prepared mix "33,35,56,68" in 3d tube of each strip. Add **15 µl** of prepared mix "51,52,58,66" in 4th tube of each strip. In case of the use of strip of 8 tubes mixes should be added analogously 1, 2, 3, 4.

NOTE: Be careful not to change the reaction mixes order in strips for adequate results processing.

Add **10 µl** of extracted DNA samples into 4 tubes with different reaction mixes.

NOTE: Ensure that the sorbent is not transferred to the PCR reaction mixture while adding DNA samples.

Control samples addition when the 72-well rotor is used

For quantitative analysis the corresponding calibrators C1 and C2 are used for each type of mixes. For this add subsequently 10 µl of C1 *HPV* 16,18,31 / Glob, C1 *HPV* 39,45,59 / Glob, C1 *HPV* 33,35,56,68, C1 *HPV* 51,52,58,66 to the tubes from 65 to 68. Add subsequently 10 µl of C2 *HPV* 16,18,31 / Glob, C2 *HPV* 39,45,59 / Glob, C2 *HPV* 33,35,56,68, C2 *HPV* 51,52,58,66 to the tubes from 69 to 72.

For qualitative analysis for carrying out the positive controls the calibrators C2 are used for each type of mixes. For this add subsequently 10 µl of C2 *HPV* 16,18,31 / Glob, C2 *HPV* 39,45,59 / Glob, C2 *HPV* 33,35,56,68, C2 *HPV* 51,52,58,66 to the tubes from 69 to 72.

Add **10 µl** of DNA sample extracted from Negative control (C–) in 4 tubes with different PCR mixes (from 61 to 64 for quantitative analysis and from 65 to 68 for qualitative analysis).

Control samples addition when the 96-well plate is used

For quantitative analysis the corresponding calibrators C1 and C2 are used for each type of mixes. For this add subsequently 10 µl of C1 *HPV* 16,18,31 / Glob, C1 *HPV* 39,45,59 / Glob, C1 *HPV* 33,35,56,68, C1 *HPV* 51,52,58,66 to the tubes from 89 to 92. Add subsequently 10 µl of C2 *HPV* 16,18,31 / Glob, C2 *HPV* 39,45,59 / Glob, C2 *HPV* 33,35,56,68, C2 *HPV* 51,52,58,66 to the tubes from 93 to 96.

For qualitative analysis for carrying out the positive controls the calibrators C2 are used for each type of mixes. For this add subsequently 10 µl of C2 *HPV* 16,18,31 / Glob, C2 *HPV* 39,45,59 / Glob, C2 *HPV* 33,35,56,68, C2 *HPV* 51,52,58,66 to the tubes from 93 to 96.

Add **10 µl** of DNA sample extracted from Negative control (C–) to 4 tubes with different PCR mixes (from 85 to 88 for quantitative analysis and from 89 to 92 for qualitative analysis).

8.2.2. Amplification

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

Table 4

AmpliSens-1 amplification program

	Rotor-type instruments [5]			Plate-type instruments [6]		
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 Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyse 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 four channels:

– The signal of *HPV* genotypes 16,39,33,58 DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of *HPV* genotypes 31,45,35,52 DNA amplification product is detected in the channel for the JOE fluorophore.
– The signal of *HPV* genotypes 18,59,68,66 DNA amplification product is detected in the channel for the ROX fluorophore.
– The signal of *HPV* genotypes 56 and 51 DNA amplification product is detected in the channel for the Cy5 fluorophore (in the tubes with PCR-mix-FL *HPV* 33,35,56,68 and 51,52,58,66, correspondingly). The signal of endogenous internal control (β-globin gene fragment) amplification product is detected in the channel for the Cy5 fluorophore in the tubes with PCR-mix-FL *HPV* 16,18,31 / Glob and 39,45,59 / Glob.

Table 5

Matrix for comparison			
FAM	**JOE**	**ROX**	**Cy5**
16	**31**	**18**	**IC**
39	**45**	**59**	**IC**
33	**35**	**68**	**56**
58	**52**	**66**	**51**

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.

Qualitative analysis

The presence of corresponding *HPV* HCR genotype is registered when the threshold *Ct* value is detected not more than boundary Ct value for the corresponding PCR mix and in the corresponding detection channel.

Results calculation and analysis is carrying out automatically with the use of software AmpliSens® HPV HCR genotype-titre in Microsoft® Excel format.

Quantitative analysis

The calibration curve is automatically plotted on the basis of the threshold *Ct* values and known calibrators values, and human DNA and each detected genotype *HPV* DNA concentrations (copies) are calculated. The obtained values are used for calculation of *HPV* DNA quantity per 100,000 human cells.

Results calculation and analysis is carrying out automatically with the use of software AmpliSens® *HPV* HCR genotype-titre in Microsoft® Excel format.

NOTE: Needed parameters for calculation are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The results are interpreted in accordance with the table 6.

Table 6

Interpretation of results lg (*HPV* per 100,000 human cells)

Result lg (*HPV* per 100,000 human cells)	Interpretation
<3	Low clinical significance
3–5	Clinically valuable. Dysplasia cannot be excluded; risk of dysplasia
>5	Clinically valuable, of increased value. Dysplasia is highly suggestive

The result of the analysis is considered reliable only if the results obtained for Positive Controls of amplification and Negative Control 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**	**Cy5**
C–	DNA extraction	Absent in all 4 tubes	Absent in all 4 tubes	Absent in all 4 tubes	Absent in all 4 tubes
C1, C2	PCR	Present in all 4 tubes	Present in all 4 tubes	Present in all 4 tubes	Present in all 4 tubes

10. TROUBLESHOOTING

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

1. Human DNA concentration value is less than 10^3 GE/reaction (the value obtained for samples in the channel for the Cy5 fluorophore in 1st and 2d sample tubes). The insufficient quantity of biological material was obtained or sample pretreatment failure occurred. The PCR analysis of this sample should be repeated beginning with the DNA extraction stage.
2. Correlation coefficient R^2 (obtained when plotting the calibration curve) is less than 0.9. The PCR analysis of all sample should be repeated beginning with the DNA extraction stage.
3. If for Negative Control of extraction (C–) any *Ct* value appears in the channels for the FAM, JOE, ROX and/or Cy5 fluorophores, it indicates the contamination of the reagents or samples. In this case results of the analysis for all samples are considered to be invalid. It is necessary to repeat the analysis of all samples, in which *HPV* DNA was detected, and to take measures to detect and eliminate the source of contamination.
4. If for PCR calibrators the *Ct* value is absent or exceeds the boundary values appears in the channels for the FAM, JOE, ROX and/or Cy5 fluorophores, it is necessary to repeat the amplification of all samples, in which *HPV* DNA was not detected.
5. If for the sample the *Ct* value is not defined or exceeds the boundary value in the channels for the FAM, JOE, ROX and/or Cy5 fluorophores in 3th and 4th tubes, and the Ct value exceeds the boundary value in the channels for the Cy5 fluorophore in 1st and 2d tubes, then the PCR analysis should be repeated beginning with the DNA extraction stage. Possible reason is a mistake of biological material preparing that leads to DNA loss, or the presence of PCR inhibitors.

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

11. TRANSPORTATION

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

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* HCR genotype-titre-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 genotype-titre-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-FL *HPV* 16,18,31 / Glob, PCR-mix-FL *HPV* 39,45,59 / Glob, PCR-mix-FL *HPV* 33,35,56,68, PCR-mix-FL *HPV* 51,52,58,66 are to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity and linear range

Biological material	Transport medium	Nucleic acid extraction kit	Sensitivity, copies/ml	Linear range of *HPV* DNA measurement, copies/ml
Urogenital swabs	Transport Medium with Mucolytic Agent	DNA-sorb-AM	1,000	1,000-100,000,000

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HPV* HCR genotype-titre-FRT** PCR kit is ensured by the 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 the DNA fragment of *HPV* genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. Analytical specificity was studied by addition of different microorganisms DNA/RNA (*human adenovirus* types 2, 3, 7, *cytomegalovirus, epstein-barr virus, varicella-zoster virus, hepatitis B virus, hepatitis C virus, human immunodeficiency virus* type 1*, human herpes virus* type 6 and 8*, herpes simplex virus, Chlamydia trahomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Neisseria gonorrhoeae, Trichomonas vaginalis, Candida albicans, Streptococcus pyogenes, Staphylococcus aureus,* DNA of *human papillomaviruses* genus β, γ, μ (1, 3, 4, 5, 8, 37, 38, 65, 20, 24, 49, 50, 15), genus α with low or unknown risk (6, 11, 26, 53, 7, 27, 10, 70, 67) in concentration 10^8 copies of *HPV* DNA per ml) in reaction. Nonspecific responses were absent.

The clinical specificity of **AmpliSens® *HPV* HCR genotype-titre-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 Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *HPV* HCR genotype-titre-FRT** PCR kit for qualitative and quantitative detection and differentiation of DNA of *human papillomaviruses* of high carcinogenic risk (*HPV* HCR) in the biological 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* HCR genotype-titre-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
19.02.16 ME	Text	Corrections according to the template, grammar corrections. AmpliSens® DNA-sorb-D nucleic acid extraction kit was added
13.02.18 ME	Text	The reference number of AmpliSens® DNA-sorb-D nucleic acid extraction kit was changed
14.09.18 EM	3. Content	The colour of the reagents was specified
17.04.20 MM	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added
	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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