



For *in Vitro* Diagnostic Use

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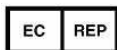
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# AmpliSens® HPV HCV screen-FEP

PCR kit

## Instruction Manual

# AmpliSens®



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## 1. INTENDED USE.

**AmpliSens® HPV HCR screen-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of high carcinogenic risk (HCR) *human papillomaviruses* (HPV) types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67 DNA in the clinical material (cervical and urethral scrapes) by means of real-time hybridization-fluorescence detection.

## 2. PRINCIPLE OF PCR DETECTION.

HPV types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In **Fluorescent End-Point** PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® HPV HCR screen-FEP** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF) that activates by heating at 95 °C for 15 min. The test is based on simultaneous real-time amplifying (multiplex PCR) and end-point detection of DNA fragments of HPV and a fragment of β-globin gene which is used as the internal endogenous control. Test identifying eleven types of HPV HCR is running either in a single tube or two tubes depending on the variant of PCR kit.

DNA-target selected as endogenous internal control is the fragment of human genome and must be present in a specimen (cervical scrape) in sufficient quantity equivalent to that of cells in the sample (no less than 10<sup>3</sup>-10<sup>5</sup> genomes). Therefore, not only does endogenous internal control allow to monitor stages of the test (DNA extraction and PCR conducting), but also to assess the adequacy of clinical material collection and storage. If the amount of epithelial cells in the specimen insufficient, amplification signal of β-globin gene will be too low.

### Detection of clinically significant virus quantity by using of **AmpliSens® HPV HCR screen-FEP** PCR kit.

According to epidemiologic studies most routine screening examinations for dysplastic changes of cervix, vagina, and vulva as well as risk of their development require detection of *clinically valuable* quantity of high carcinogenic risk human papillomavirus. Believed, that detection of virus in quantity not exceeding certain threshold value is clinically insignificant because 100% of such cases associates with spontaneous recovery. On the contrary, high virus load suggests about dysplasia or risk of its development. However, in case of monitoring of treatment, diagnosis of even low virus load can marker an early relapse. Currently, level of clinically significant virus quantity estimates at 10<sup>5</sup> GE of HCR HPV per cervical scrape when standardized obtaining of clinical material is provided. Clinical investigations done on model clinical samples have showed that only clinically significant virus quantity is detected if following steps are applied:

- collection of cervical scrape by standard procedure ( placed in 0.5 ml of transport medium)
- DNA extraction (DNA-sorb-AM were used);
- 100x dilution of obtained DNA in TE-buffer;
- PCR-test.

Clinical trials of this approach on specimens collected from both healthy patients and patients suffering from

severe dysplasia and cervical cancer demonstrated increase of specificity of dysplasia detection by 22.9% (from 61.7% without dilution to 84.6% if dilution was applied) while high level of severe dysplasia and cervical cancer diagnosis was maintained (98.9%). Note that level of clinically significant virus quantity wasn't validated for men.

Therefore, **AmpliSens® HPV HCR screen-EPh** PCR kit allows two formats of HCR HPV detection:

- presence of HPV HCR (sample to be tested after DNA extraction)
- clinically significant quantity of HPV HCR (sample to be tested after DNA extraction and dilution in TE-buffer). Note that standardized obtaining of clinical material is necessary.

## 3. CONTENT.

**AmpliSens® HPV HCR screen-FEP** PCR kit is produced in 2 forms:

AmpliSens® HPV HCR screen-FEP PCR kit variant screen-FEP 2x **REF** V31-FEP-CE.

AmpliSens® HPV HCR screen-FEP PCR kit variant screen-FEP 3x **REF** V31-3x-FEP-CE.

AmpliSens® HPV HCR screen-FEP PCR kit variant screen-FEP 2x includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP HPV-1 (per 30 reactions)	colorless, clear liquid	0.21	4 blue cap tubes
PCR-mix-1-FEP HPV-2 (per 30 reactions)	colorless, clear liquid	0.21	4 green cap tubes
PCR-buffer-Flu (per 56 reactions)	colorless, clear liquid	0.42	4 tubes
Polymerase (TaqF) (per 56 reactions)	colorless, clear liquid	0.028	4 tubes
PCR-mix-Background HPV	colorless, clear liquid	0.8	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 vial
Positive Control DNA HPV types 16, 31, 33 and human DNA	colorless, clear liquid	0.2	2 tubes
TE-buffer	colorless, clear liquid	5.0	5 tubes
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction.

AmpliSens® HPV HCR screen-FEP PCR kit is intended for 120 reactions, including controls.

AmpliSens® HPV HCR screen-FEP PCR kit variant screen-FEP 3x includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP HPV 3x (per 30 reactions)	colorless, clear liquid	0.21	4 liquid
PCR-buffer-Flu (per 56 reactions)	colorless, clear liquid	0.42	2 liquid
Polymerase (TaqF) (per 56 reactions)	colorless, clear liquid	0.028	2 liquid
PCR-mix-Background HPV	colorless, clear liquid	0.8	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 vial
Positive Control DNA HPV types 16, 31, 33 and human DNA	colorless, clear liquid	0.2	1 tube
TE-buffer	colorless, clear liquid	5.0	5 tubes
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction.

AmpliSens® HPV HCR screen-FEP PCR kit is intended for 120 reactions, including controls.

## 4. ADDITIONAL REQUIRMENTS.

- DNA isolation kit.

- Disposable powder-free gloves.
- 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.
- Personal thermocyclers (for example, Gradient Palm Cyclers (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), Terzik (DNA-Technology, Russia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature below minus 16 °C.
- Waste bin for used tips.

## 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which 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.



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

**AmpliSens® HPV HCR screen-FEP** PCR kit is intended for the analysis of DNA extracted with DNA isolation kits from:

– *cervical or urethral scrapes.*

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

**Method 1** - use the sampling kit which includes one/two cervical cytobrushes and 2 ml tube with 0.5 ml of

transport medium with mucolytic "TSM" [REF](#) 953.

Endocervical epithelial scrape, obtained with first cytobrush and/or exocervical epithelial scrape obtained with second cytobrush should be placed into the tube with transport media. Break the effective part of the cytobrushes with the sample at the score mark and leave them in the tube.

**Method 2** - use "Digene" (USA) sampling kit, which contains cervical cytobrush and 1.0 ml tube with "Digene" transport medium.

Endocervical epithelial scrape obtained with cytobrush should be placed into the tube with "Digene" transport medium.

**Method 3** - use the sampling kit, which contains combined gynecological probe for simultaneous obtaining of epithelial cells from endo-/exocervix and 5 ml tube with 2.0 ml of transport medium with mucolytic "TSM" [REF](#) 953.

Place endocervical and exocervical epithelial scrapes into the tube with transport medium. Break the effective part of the cytobrush with the sample at the score mark and leave it in the tube.

**Method 4** - use "CytoScreen" (Italy) or "PreservCyt" (USA) sampling kits which contain combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and a vial with transport-fixation medium.

Place endocervical and exocervical epithelial scrapes into the tube with transport-fixation medium. Break the effective part of the cytobrush with the sample at the score mark and leave it in the vial.

**Male:** Obtain urethral epithelial scrape by universal probe, place it into the 2.0 ml tube with 0.5 ml of transport medium with mucolytic "TSM" [REF](#) 953.

Storage of the samples:

- between 18 and 25 °C up to 5 days;
- between 2 and 8 °C up to 20 days;
- at minus 16 °C or below for 1 year.



Only one freeze-thaw cycle of clinical material is allowed.

## 7. PROTOCOL.

### 7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-AM", [REF](#) K1-12-100-CE (for clinical material obtained by methods 1, 2,3);
- "DNA-sorb-B", [REF](#) K1-2-100-CE (for clinical material obtained by methods 1, 2, 3);
- "DNA-sorb-C", [REF](#) K1-6-50-CE (for biopsy materials);

### 7.2. Preparing of the PCR. **VARIANT SCREEN-FEP 2x**

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

**This variant applies two mixes of primers and probes:**


– **PCR-mix-1-FEP HPV-1** is intended for amplification and detection of HPV genotypes 16, 31, 35, 39 and 59 DNAs. Genotypes 31, 35, 39 and 59 are detected in the FAM channel (channel 1 of ALA-1/4 fluorescence

detector). Genotype 16 is detected separately in HEX/JOE channel (channel 2 of ALA-1/4 fluorescence detector). This mix doesn't contain Internal Control.

— **PCR-mix-1-FEP HPV-2** is intended for amplification and detection of HPV genotypes 18, 33, 45, 52, 58 and 67 DNAs as well as the fragment of human  $\beta$ -globin gene (internal endogenous control). Genotypes 18, 33, 45, 52, 58 and 67 are detected in the FAM channel (channel 1 of ALA-1/4 fluorescence detector). Internal endogenous control is detected separately in HEX/JOE channel (channel 2 of ALA-1/4 fluorescence detector). DNA amplification should be performed by applying both PCR-mix-1-FEP HPV-1 and PCR-mix-1-FEP HPV-2. The result is considered positive if positive signal is recorded at least for one of the PCR-mixes-1-FEP.

### 7.2.1 Preparing of tubes for PCR.

1. Prepare **PCR-buffer-Flu** and **polymerase (TaqF)** mix. To do this, transfer the content of one tube with polymerase (TaqF) (28  $\mu$ l) to the tube with PCR-buffer-Flu (420  $\mu$ l) and carefully vortex. Avoid foaming while mixing. Indicate the time of the mix preparation on the tube.

 Prepared mix is intended for **56** reactions. The mix should be stored from 2 to 8 °C for 3 months and used as needed.

2. Prepare reaction mixes (see table 1). When calculating take into account that every run should include two controls for each mix (**negative and positive controls**). Moreover, it is necessary to add reagents for one spare reaction.

3. Each PCR reaction should include:

- **7  $\mu$ l of PCR-mix-1-FEP HPV-1 or PCR-mix-1-FEP HPV-2;**
- **8  $\mu$ l of PCR-buffer-Flu and polymerase (TaqF) mix.**

Table 1

**Reaction mixes preparation scheme**

Number of samples	1	2	3	4	5	6	7	8	9	10	11	12	13
PCR-mix-1-FEP HPV-1/ HPV-2, $\mu$ l	28	35	42	49	56	63	70	77	84	91	98	105	112
PCR-buffer-Flu and polymerase (TaqF) mix, $\mu$ l	32	40	48	56	64	72	80	88	96	104	112	120	128
Number of samples	14	15	16	17	18	19	20	21	22	23	24	25	26
PCR-mix-1-FEP HPV-1/ HPV-2, $\mu$ l	119	126	133	140	147	154	161	168	175	182	189	196	203
PCR-buffer-Flu and polymerase (TaqF) mix, $\mu$ l	136	144	152	160	168	176	184	192	200	208	216	224	232

Calculation for PCR-mix-1-FEP HPV-1 is the same as for PCR-mix-1-FEP HPV-2.

4. Insert in a tube rack two PCR-tubes for each clinical sample, two tubes for positive control, and two tubes for negative control. Transfer 15  $\mu$ l of reaction mix, containing PCR-mix-1-FEP HPV-1 per half of the tubes (per each

tube). To the remaining half of the tubes add 15  $\mu$ l of reaction mix, containing PCR-mix-1-FEP HPV-2 (per each tube).


5. Add above 1 drop of mineral oil for PCR.

6. Prepare **2 background samples per each PCR-mix-1.**

— **If one of the recommended DNA extraction kits is used**, to two PCR tubes transfer 7  $\mu$ l of PCR-mix-1-FEP HPV-1 and 18  $\mu$ l of PCR-mix-Background HPV (per each). Add above 1 drop of mineral oil for PCR.

— **If different way is applied for DNA extraction**, to two PCR tubes transfer 8  $\mu$ l of PCR-mix-1-FEP HPV-1, 8  $\mu$ l of PCR-buffer-Flu, and 10  $\mu$ l of Negative Control of Extraction (C-) (per each). Add above 1 drop of mineral oil for PCR.

Prepare background samples for PCR-mix-1-FEP HPV-2 similarly.

 Background samples, that have once passed thermal cycling, can be used for further runs if stored between 2 and 20 °C for up to 1 month. Multiple use of Background samples is permitted only if the same lot of the PCR kit, the same extraction kit, and the same type of PCR tubes are applied.

7. Add **10  $\mu$ l of DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared pair of tubes.

8. Carry out control amplification reactions:

**NCA** Add **10  $\mu$ l of TE-buffer** to the pair of tubes labeled NCA (Negative Control of Amplification).

**C+** Add **10  $\mu$ l of Positive Control DNA HPV types 16, 31, 33 and human DNA** to the pair of tubes labeled C+ (Positive Control of Amplification).

### 7.2.2 Amplification.

Run the following program on the thermocycler (see Table 2). When the temperature will reach 95°C (pause regimen), insert tubes into the cells of amplifier and press the button to continue. It is recommended to sediment drops from walls of tubes by short vortex (2–3 sec) before their insertion in a thermal cycler.

Table 2

**Programming thermocycler for DNA amplification of HPV types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67**

step	Thermocyclers with active temperature adjustment:									Other thermocyclers		
	"Tercik" (DNA technology)			"GeneAmp PCR System 2700" (ABI)			"Gradient Palm Cycler" (Corbett Research), "Maxygene" (Axygen)					
	tempera ture	time	cycles	tempera ture	time	cycles	tempera ture	time	cycles	tempera ture	time	cycles
1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15 min	1
2	93 °C	5 sec	20	95 °C	10 sec	50	93 °C	5 sec	50	95 °C	25 sec	50
	59 °C	5 sec		59 °C	20 sec		59 °C	10 sec		59 °C	25 sec	
3	72 °C	5 sec	30	72 °C	10 sec	1	72 °C	5 sec	1	72 °C	25 sec	1
	93 °C	2 sec		72 °C	1 min		72 °C	1 min		72 °C	1 min	
4	59 °C	10 sec	1	95 °C	20 sec	1	95 °C	20 sec	1	95 °C	20 sec	1
	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	

For results interpretation refer to section 8.1.

### 7.3. Preparing of the PCR. **VARIANT SCREEN-FEP 3x**

Total reaction volume is **25  $\mu$ l**, the volume of DNA sample is **10  $\mu$ l**.

**This variant applies a single mixture of primers and probes:**

— Genotypes 31, 35, 39 and 59 are detected in the ROX channel (channel 3 of ALA-1/4 fluorescence detector). Genotypes 16, 18, 33, 45, 52, 58 and 67 are detected in FAM channel (channel 1 of ALA-1/4 fluorescence detector), Internal Control is detected separately in HEX channel (channel 2 of ALA-1/4 fluorescence detector).

**7.3.1 Preparing tubes for PCR.**

1. Prepare the mixture of **PCR-buffer-Flu** and **polymerase (TaqF)**. To do this, transfer the content of one tube with polymerase (TaqF) (28 µl) to the tube with PCR-buffer-Flu (420 µl) and carefully vortex. Avoid foaming while mixing. Indicate the time of the mix preparation on the tube.



The mix should be stored between 2 and 8 °C for 3 months and used as needed.

2. Mix following reagents in a separate tube calculating per one reaction:

— **7 µl of PCR-mix-1-FEP HPV 3x;**

— **8 µl of mix of PCR-buffer-Flu and polymerase (TaqF).**

When calculating take into account that every run should include two controls (**negative and positive**). Moreover, it is necessary to add reagents for one spare reaction.

3. Insert in a tube rack one PCR-tube for each clinical sample one tube for positive control and one tube for negative control. Transfer **15 µl** of reaction mix per each tube.

4. Add above 1 drop of mineral oil for PCR.

5. Prepare **2 background samples**:

— **If one of the recommended DNA extraction kits is used**, transfer 7 µl of PCR-mix-1-FEP HPV 3x and 18 µl of PCR-mix-Background HPV to two PCR tubes (per each tube). Add above 1 drop of mineral oil for PCR.

— **If different way is applied for DNA extraction**, transfer 8 µl of PCR-mix-1-FEP HPV 3x, 8 µl of PCR-buffer-Flu, and 10 µl of Negative Control of Extraction (C-) to two PCR tubes (per each tube). Add above 1 drop of mineral oil for PCR.



Background samples, that have once passed thermal cycling, can be used for further runs if stored between 2 and 20 °C for up to 1 month. Multiple use of Background samples is permitted only if the same lot of the PCR kit, the same extraction kit, and the same type of PCR tubes are applied.

6. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared pair of tubes.

7. Carry out control amplification reactions:

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

**C+** Add **10 µl** of **Positive Control DNA HPV types 16, 31, 33 and human DNA** to the tube labeled C+ (Positive Control of Amplification).

**7.3.2 Amplification.**

Run the amplification program on the thermocycler (see Table 3). When the temperature will reach 95°C (pause regimen), insert tubes into the cells of amplifier and press the button to continue. It is recommended to sediment drops from walls of tubes by short vortex (2–3 sec) before their insertion in a thermocycler.

Table 3

**Programming thermocycler for DNA amplification of HPV types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67**

step	Thermocyclers with active temperature adjustment:									Other thermocyclers		
	"Tercik" (DNA technology)			"GeneAmp PCR System 2700" (ABI)			"Gradient Palm Cycler" (Corbett Research), "Maxygene" (Axygen)					
	tempera- ture	time	cycles	tempera- ture	time	cycles	tempera- ture	time	cycles	tempera- ture	time	cycles
1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15 min	1
2	93 °C	5 sec	20	95 °C	10 sec	50	93 °C	5 sec	50	95 °C	25 sec	50
	59 °C	5 sec		59 °C	20 sec		59 °C	10 sec		59 °C	25 sec	
	72 °C	5 sec		72 °C	10 sec		72 °C	5 sec		72 °C	25 sec	
3	93 °C	2 sec	30	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
	59 °C	10 sec		95 °C	20 sec	1	95 °C	20 sec	1	95 °C	20 sec	1
4	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	

For results interpretation refer to section 8.2.

**8. DATA ANALYSIS.**

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 1 or Appendix 2 (for variant screen-FEP 2x or variant screen-FEP 3x, respectively).



Detection can be conducted within 1 week from the end of the amplification only if the tubes with the amplified product were stored from 2 to 20°C.

**8.1. Results interpretation. VARIANT SCREEN-FEP 2x**

**Results interpretation for "HPV-1" mix (HPV1 test)**

This mix allows detecting of part of HPV HCR genotypes (FAM channel) and separately identifies genotype 16 (HEX channel). Correspondently, if positive result is registered in FAM channel it indicate "HPV HCR is detected" result, if positive result is registered in HEX channel it indicate "HPV genotype 16 is detected" result.

PCR-mix-1-FEP HPV-1 doesn't include Internal Control. So, if negative result is obtained for both channels the complete result of the test (negative or invalid) will be determined by results for "HPV-2" mix.

**Results interpretation for "HPV-2" mix (HPV2 test)**

This mix allows detecting of the other part of HPV HCR genotypes (FAM channel) and Internal Control (HEX channel). Correspondently, if positive result is registered in FAM channel, it indicate "HPV HCR is detected" result.

Negative result in FAM channel and positive result in HEX indicate "HPV HCR is not detected" result. Negative signal in both, FAM and HEX, channels indicate "Invalid" result. However, even if invalid result is defined for "HPV-2" mix, total analysis result can be positive in case HPV HCR or HPV type 16 are found in "HPV-1" mix (see table 4).

Table 4

Interpretation of total analysis results

"HPV-1" mix (HPV1)		"HPV-2" mix (HPV2)		Result
FAM (HPV HCR)	HEX (HPV 16)	FAM (HPV HCR)	HEX (IC)	
-	-	-	+	HPV HCR is not detected
+	-	-	+	HPV HCR is detected
-	+	+	+	HPV type 16 is detected
+	+	-	+	HPV HCR, including type 16 is detected
-	-	+	-	Invalid result
+	-	-	-	HPV HCR is detected
-	+	+	-	HPV type 16 is detected
+	+	-	-	HPV HCR including type 16 is detected
-	+	+	-	
+	+	+	-	

Result is accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed (see table 5).

Table 5

Results for controls

Control	Stage for control	Results of automatic interpretation		Interpretation
		Result for PCR-mix-1-FEP HPV-1	Result for PCR-mix-1-FEP HPV-2	
C-	DNA isolation	FAM channel <i>negative</i> HEX channel <i>negative</i>	FAM channel <i>negative</i> HEX channel <i>negative</i>	OK
NCA	Amplification	FAM channel <i>negative</i> HEX channel <i>negative</i>	FAM channel <i>negative</i> HEX channel <i>negative</i>	OK
C+	Amplification	FAM channel <i>positive</i> HEX channel <i>positive</i>	FAM channel <i>positive</i> HEX channel <i>positive</i>	OK

## 8.2. Results interpretation. VARIANT SCREEN-FEP 3X

1. When the analysis is complete the results are automatically shown in the table as follows:

**pos** – positive result;

**neg** – negative result;

**eq** – equivocal result (signal at the channel for detection of specific cDNA exceed threshold value for negative samples, but does not exceed threshold value for positive samples (signal is in grey zone);

**nd** – invalid result (specific signal and IC signal does not detect (does not exceed threshold value) in the sample).

2. Result is accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed (see table 6).

Table 6

Results for controls

Control	Stage for control	Result of automatic interpretation	Interpretation
C-	DNA isolation	FAM channel <i>negative</i> HEX channel <i>negative</i> ROX channel <i>negative</i>	OK
NCA	Amplification	FAM channel <i>negative</i> HEX channel <i>negative</i> ROX channel <i>negative</i>	OK
C+	Amplification	FAM channel <i>positive</i> HEX channel <i>positive</i> ROX channel <i>positive</i>	OK

## 9. TROUBLESHOOTING.

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

## 10. STABILITY AND STORAGE.

All components of the **AmpliSens® HPV HCR screen-FEP** PCR kit (except for polymerase (TaqF), PCR-mix-1-FEP HPV-1, PCR-mix-1-FEP HPV-2, and PCR-mix-1-FEP HPV 3x) are to be stored between 2°C and 8°C, when not in use. All components must be stable until the expiry date stated on the label.



Polymerase (TaqF), PCR-mix-1-FEP HPV-1, PCR-mix-1-FEP HPV-2, and PCR-mix-1-FEP HPV 3x are to be stored at not more than minus 16 °C. Do not expose PCR-mix-1-FEP HPV-1, PCR-mix-1-FEP HPV-2, and PCR-mix-1-FEP HPV 3x to light for a long period of time.

## 11. SPECIFICATIONS.

### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® HPV HCR screen-FEP** PCR kit is no less than  $1 \times 10^3$  genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® HPV HCR screen-FEP** PCR kit are guaranteed only when additional reagents kit, "DNA-sorb-AM", "DNA-sorb-B", or "DNA-sorb-C" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), is used.

### 11.2. Specificity.

Specificity of **AmpliSens® HPV HCR screen-FEP** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® HPV HCR screen-FEP** PCR kit was confirmed in laboratory clinical trials.

















## 12. REFERENCES.

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

### 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens® HPV HCR screen-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

### 14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control