CE

IVD

For Professional Use Only

AmpliSens® *HPV* HCR screen-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

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 using end-point hybridization-fluorescence detection.

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

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 **F**luorescent **E**nd-**P**oint 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 ºC 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 10^3-10^5 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 10^5 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 was 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-FEP** 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 (C+$_{HPV\ 16,31,33}$)	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 tubes
PCR-buffer-Flu (per 56 reactions)	colorless clear liquid	0.42	2 tubes
Polymerase (TaqF) (per 56 reactions)	colorless clear liquid	0.028	2 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 (C+$_{HPV\ 16,31,33}$)	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 Cycler (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 (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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:

– c*ervical 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-CE.

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-CE.

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-CE.

Storage of the samples:

- at 18-25 °C up to 5 days;
- at 2-8 °C up to 20 days;
- at ≤ –16 °C for 1 year.

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

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR screen-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. 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);

8.2. 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 β-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.

8.2.1 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 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.

Prepared mix is intended for **56** reactions. The mix should be stored at 2-8 °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 µl of PCR-mix-1-FEP *HPV*-1 or PCR-mix-1-FEP *HPV*-2;**
   - **8 µl 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, µl	28	35	42	49	56	63	70	77	84	91	98	105	112
PCR-buffer-Flu and polymerase (TaqF) mix, µl	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, µl	119	126	133	140	147	154	161	168	175	182	189	196	203
PCR-buffer-Flu and polymerase (TaqF) mix, µl	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 µl 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 µl 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 µl of PCR-mix-1-FEP *HPV*-1 and 18 µl 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 µl of PCR-mix-1-FEP *HPV*-1, 8 µl of PCR-buffer-Flu, and 10 µl 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 at 2-20 ºC 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 µl** 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 µl** of **TE-buffer** to the pair of tubes labeled NCA (Negative Control of Amplification).

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

8.2.2 Amplification.

Run the following program on the thermocycler (see Table 2). When the temperature will reach 95 ºC (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 s) 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

	Thermocyclers with active temperature adjustment:									Other termocyclers		
	Tercik (DNA technology)			**GeneAmp PCR System 2700 (Applied Biosystems)**			**Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**					
step	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 s	20	**95 °C**	10 s	50	**93 °C**	5 s	50	**95 °C**	25 s	50
	59 °C	5 s		**59 °C**	20 s		**59 °C**	10 s		**59 °C**	25 s	
	72 °C	5 s		**72 °C**	10 s		**72 °C**	5 s		**72 °C**	25 s	
3	**93 °C**	2 s	30	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
	59 °C	10 s		**95 °C**	20 s	1	**95 °C**	20 s	1	**95 °C**	20 s	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

For results interpretation refer to section 8.1.

8.3. Preparing of the PCR. VARIANT SCREEN-FEP 3X

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

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).

8.3.1 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 at 2-8 °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 at 2-20 ºC 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).

8.3.2 Amplification.

Run the amplification program on the thermocycler (see Table 3). When the temperature will reach 95 ºC (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 s) 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

	Thermocyclers with active temperature adjustment:									Other termocyclers		
	Tercik (DNA technology)			**GeneAmp PCR System 2700 (Applied Biosystems)**			**Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**					
step	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 s	20	**95 °C**	10 s	50	**93 °C**	5 s	50	**95 °C**	25 s	50
	59 °C	5 s		**59 °C**	20 s		**59 °C**	10 s		**59 °C**	25 s	
	72 °C	5 s		**72 °C**	10 s		**72 °C**	5 s		**72 °C**	25 s	
3	**93 °C**	2 s	30	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
	59 °C	10 s		**95 °C**	20 s	1	**95 °C**	20 s	1	**95 °C**	20 s	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

For results interpretation refer to section 8.2.

9. DATA ANALYSIS

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

Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Guidelines

Detection can be conducted within 1 week from the end of the amplification only if the tubes with the amplified product were stored at 2- 20ºC.

9.1 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 (*HPV*1)		**"*HPV*-2" mix (*HPV*2)**		**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 *negative* HEX channel *negative*	FAM channel *negative* HEX channel *negative*	OK
NCA	Amplification	FAM channel *negative* HEX channel *negative*	FAM channel *negative* HEX channel *negative*	OK
C+	Amplification	FAM channel *positive* HEX channel *positive*	FAM channel *positive* HEX channel *positive*	OK

9.2 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 *negative* HEX channel *negative* ROX channel *negative*	OK
NCA	Amplification	FAM channel *negative* HEX channel *negative* ROX channel *negative*	OK
C+	Amplification	FAM channel *positive* HEX channel *positive* ROX channel *positive*	OK

10. TROUBLESHOOTING

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

11. TRANSPORTATION

AmpliSens® *HPV* HCR screen-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. 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 at 2–8 ºC when not in use. All components must be 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.

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 temperature from minus 24 to minus 16 °C when not in use.
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.

PCR-mix-1-FEP *HPV*-1, PCR-mix-1-FEP *HPV*-2, and PCR-mix-1-FEP *HPV* 3x are to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *HPV* HCR screen-FEP** PCR kit is no less than 1 x 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 Budget Institute of Science “Central Research Institute for Epidemiology”), is used.

13.2. Specificity

Specificity of **AmpliSens® 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.

14. REFERENCES

1. Handbook “Sampling, transportation, 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, Moscow, 2008.

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 **AmpliSens® *HPV* HCR screen-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
28.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mix-1-FEP *HPV*-1, PCR-mix-1-FEP *HPV*-2, and PCR-mix-1-FEP *HPV* 3x are to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
22.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
21.12.12 LA	3. Content	Short designation of the positive control of amplification was corrected: $C+_{HPV\ 16,31,33}$ instead of $C+_{16,31,33}$