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For Professional Use Only

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

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of DNA of *human papillomaviruses* (*HPV*) of high carcinogenic risk (HCR) in biological material (swab of vaginal mucosa, scraping of membrane mucosa of cervix uteri and urethra, endocervical scraping, biopsy material of cervical mucous membrane) by using real-time hybridization-fluorescence detection.

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 founded 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 (IC) control.

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.

In the provided test the concentration of *HPV* DNA is calculated as the relation between the number of the *HPV* genomes and human mucosal cells. For this purpose, primers and probes for human β-globin gene are present in the PCR-mix together with primers and probes for *HPV,* and the calibrators of human DNA are present in the DNA-calibrators' solutions together with the *HPV* calibrators. Relative concentration values of *HPV* DNA to human DNA, obtained in such a way, may reflect the density of bacterial content of mucosal cells by these microorganisms. Moreover, human DNA is used as an internal

endogenous control to reflect the quality of sampling the biological material.

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit 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.

3. CONTENT

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit variant FRT-100 F, **REF** R-V31-F-CE.

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
PCR-mix- FL *HPV* 14	colorless clear liquid	1.2	1 tube
PCR-buffer-B	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
DNA calibrator C1 *HPV* 16,18,45 / Glob	colorless clear liquid	0.2	1 tube
DNA calibrator C2 *HPV* 16,18,45 / Glob	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.

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit variant FRT-100 F is intended for 110 reactions, including controls.

PCR kit also includes:

- Compact Disk with: software (Microsoft® Excel format) for data interpretation and result analysis obtaining;

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.

- PCR box.
- Real-time instruments with 5 (or more) independent detection channels (for example, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  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) 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 ≤ –16 °C.
- Reservoir for disposed tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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

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.

AmpliSens® *HPV* HCR screen-titre-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material (scraping of membrane mucosa of cervix uteri and urethra, endocervical scraping, swab of vaginal mucosa, biopsy material of cervical mucous membrane):

Biological material:

For women: epithelial samples are taken in the same way as for cytological analysis:

Method 1. The sampling kit consists of one or two cervical cytobrushes 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) taken with the first cervical cytobrush and/or the superficial cervical swab (ectocervix) taken with the second cervical cytobrush to the tube with transport medium. The working part of the cytobrush is to be broken off and left in the tube with transport medium.

Method 2. The sampling kit (Digene, USA) consists of a cervical cytobrush and a tube with 1.0 ml of Digene transport medium.

Place the cervical epithelial swab (endocervix) taken with the cervical cytobrush to the tube with Digene transport medium.

Method 3. The sampling kit consists of a combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix and a 2-ml tube with 0.5 ml 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. The working part of the probe is to be broken off and left in the tube with the transport medium.

Method 4. The sampling kit consists of a combined gynecological probe for

simultaneously taking epithelial samples from endocervix and ectocervix and a jar with transport–fixation medium for fluid cytology purchased from CytoScreen (Italy) or PreservCyt (USA).

Place the cervical epithelial swab (endocervix) and the superficial cervical swab (ectocervix) into the tube with transport–fixation medium. The working part of the probe is to be broken off and left in the tube with the medium.

For men: Place the urethral epithelial swab taken with a universal probe to a 2.0-ml tube with 0.5 ml 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.

Material in the transport and fixing environment for liquid cytology is stored at room temperature throughout the year.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR screen-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.

Extract DNA according to the manufacturer's instruction.

8.2. Preparing PCR

8.2.1. Preparing tubes for.

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

1. Thaw the tube with **PCR-mix-FL *HPV* 14**. Vortex the tubes with **PCR-mix-FL *HPV* 14, PCR-buffer-B, polymerase (TaqF)** and then centrifuge briefly.

   Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.

2. For N reactions, add to a new tube:

   10x(N+1) µl of PCR-mix-FL *HPV* 14,

   5,0x(N+1) µl of PCR-buffer-B

   0,5x(N+1) µl of polymerase (TaqF).

   Vortex the tube, then centrifuge it briefly. Transfer **15 µl** of the prepared mixture to each tube.
3. Add **10 µl** of **DNA samples** extracted from test or control samples to the prepared tubes.
4. Carry out the control amplification reactions:

   C1 – Add **10 µl** of **DNA calibrator C1 *HPV* 16,18,45 / Glob** to 2 tubes

   C2 – Add **10 µl** of **DNA calibrator C2 *HPV* 16,18,45 / Glob** to 2 tubes

   C- – Add **10 µl** of **the sample extracted from the Negative Control reagent**

8.3.2. Amplification

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

Table 1

Amplification program common for rotor-[1)] and plate-type[2] instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycles*
Hold 1	50	15 min	–	1
Hold 2	95	15 min	–	1
Cycling 1	95	10 s	–	45
	60	20 s	+	

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

2. Insert tubes into the reaction module of the device.

It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.

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

[1)] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany), or equivalent.
[2)] For example, CFX96 (Bio-Rad, USA), or equivalent.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in five channels:

Channel for the fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
The registration of signal which indicates the accumulation of amplified products	DNA of *HPV* genotype 16	DNA of *HPV* genotype 18	DNA of *HPV* HCR (genotypes 16,18,31,33,35,39,45, 51,52,56,58,59,66,68)	IC β-globin DNA	DNA of *HPV* genotype 45

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 cDNA sample in the corresponding column of the results grid.

Qualitative analysis

Principle of interpretation is the following:

Table 2

Results interpretation of test samples

FAM	JOE	ROX	Cy5	Cy5.5	Result
		***Ct* value**			
Absent or >boundary value	Absent or >boundary value	**<**boundary value	**<**boundary value	Absent or >boundary value	*HPV* HCR DNA **is detected**
<boundary value	Absent or >boundary value	**<**boundary value	**<**boundary value	Absent or >boundary value	*HPV* HCR DNA **is detected** (including DNA of genotype 16)
Absent or >boundary value	**<**boundary value	**<**boundary value	**<**boundary value	Absent or >boundary value	*HPV* HCR DNA **is detected** (including DNA of genotype 18)
Absent or >boundary value	Absent or >boundary value	**<**boundary value	**<**boundary value	**<**boundary value	*HPV* HCR DNA **is detected** (including DNA of genotype 45)
Absent or >boundary value	Absent or >boundary value	Absent or >boundary value	**<**boundary value	Absent or >boundary value	*HPV* HCR DNA **is NOT detected**
Absent or >boundary value	Absent or >boundary value	Absent or >boundary value	Absent or >boundary value	Absent or >boundary value	**Invalid***

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

Results analysis is carrying out automatically with the use of software in Microsoft® Excel format.

The result of <u>qualitative analysis</u> is considered reliable only if the results obtained for Positive Controls of amplification and Negative Control of extraction are correct (see Table 3)

Table 3

Results for controls

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

<u>Quantitative analysis</u>

The calibration curve is automatically plotted on the basis of the threshold *Ct* values and known calibrators (C1 and C2) values, and human DNA and *HPV* DNA concentrations (copies) are calculated. Obtained data are used for calculation of *HPV* DNA quantity per 100.000 human cells according to the formula:

$$\lg\left(\frac{\text{number of } HPV \text{ DNA copies in PCR-sample}}{\text{number of human DNA copies in PCR-sample}} \times 2*10^5\right) = \lg(HPV \text{ DNA copies } /10^5 \text{ cells})$$

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

Results calculation and analysis is carrying out automatically with the use of software in Microsoft® Excel format.

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

Obtained result is interpreted in accordance with the Table 4:

Table 4

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

Result lg (*HPV* per 100,000 human cells)	Interpretation
<3	Clinically insignificant value
3–5	Clinically significant value. Dysplasia cannot be excluded; risk of dysplasia development
>5	Clinically significant, increased value. High probability of dysplasia
Integration? (only for genotypes 16, 18 and 45)	Identification of E6 area in the absence of E1/E2 area indirectly suggests the probability of viral integration into the human DNA.

The result is **invalid** if the concentration value of human DNA (obtained for samples in the

channel for Cy5 fluorophore) is less than 10^3 copies/reaction and the calculated concentration values are absent in the channels for FAM, JOE, ROX, Cy5.5 fluorophores. It is necessary to repeat the PCR analysis of this sample starting from DNA extraction stage. If human DNA is absent in the test sample, it is recommended to repeat biological material sampling and PCR-analysis.

The results of the <u>quantitative analysis</u> is considered reliable only if the results obtained for Positive Controls of amplification and Negative Control of extraction are correct (see Table 5)

Table 5

Results for controls

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

10. TROUBLESHOOTING

1. The positive result was obtained for test sample, while the fluorescence curve has not the typical exponential growth (the fluorescence curve is approximately like a straight line). Such result couldn't be considered as positive, it is necessary to repeat the PCR-analysis for this sample.
2. If the *Ct* value is determined for Negative Control of Extraction in any of 5 channels for FAM, JOE, ROX, Cy5, Cy5.5 fluorophores, the contamination of laboratory by amplified products or contamination of reagents, test samples at any stage of the analysis is possible. It is necessary to take measures to detect and eliminate the contamination source and to repeat PCR-analysis (starting from DNA-extraction stage) of all samples, in which specific DNA was detected.
3. Correlation coefficient R^2 (obtained when plotting the calibration curve) for quantitative analysis is less than 0.98. The PCR analysis of all samples should be repeated.
4. If the Ct value is absent for DNA-calibrators C1 and C2 in any of the channels for the FAM, JOE, ROX, Cy5, Cy5.5 fluorophores, the PCR analysis should be repeated for all the samples.

11. TRANSPORTATION

AmpliSens® *HPV* HCR screen-titre-FRT 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-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 screen-titre-FRT** PCR kit are stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

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

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Transport medium	DNA-extraction kit	*HPV* genotype	Analytical sensitivity, copies/ml
Swab of vaginal mucosa, scraping of membrane mucosa of cervix uteri and urethra, endocervical scraping	Transport medium for swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM	16	$1x10^3$
			18	$1x10^3$
			31	$1x10^3$
			33	$1x10^3$
			35	$1x10^3$
			39	$1x10^3$
			45	$1x10^3$
			51	$1x10^3$
			52	$1x10^3$
			56	$1x10^3$
			58	$1x10^3$
			59	$1x10^3$
			66	$1x10^3$
			68	$1x10^3$

The claimed sensitivity is achieved only when biomaterial pretreatment is carried out in accordance with the chapter *Sampling and Handling.*

The analytical sensitivity for each microorganism is preserved in the presence of high DNA concentrations of other analyte microorganism (10^8 GE/ml).

Linear measurement range and detection limit

Biological material	Transport medium	DNA-extraction kit	*HPV* genotype	Detection limit, copies/ml	Linear measurement range, copies/ml
Swab of vaginal mucosa, scraping of membrane mucosa of cervix uteri and urethra, endocervical scraping	Transport medium for swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM	16	$1x10^3$	$1x10^3 – 1x10^8$
			18	$1x10^3$	$1x10^3 – 1x10^8$
			31	$1x10^3$	$1x10^3 – 1x10^8$
			33	$1x10^3$	$1x10^3 – 1x10^8$
			35	$1x10^3$	$1x10^3 – 1x10^8$
			39	$1x10^3$	$1x10^3 – 1x10^8$
			45	$1x10^3$	$1x10^3 – 1x10^8$
			51	$1x10^3$	$1x10^3 – 1x10^8$
			52	$1x10^3$	$1x10^3 – 1x10^8$
			56	$1x10^3$	$1x10^3 – 1x10^8$
			58	$1x10^3$	$1x10^3 – 1x10^8$
			59	$1x10^3$	$1x10^3 – 1x10^8$
			66	$1x10^3$	$1x10^3 – 1x10^8$
			68	$1x10^3$	$1x10^3 – 1x10^8$

The claimed values of characteristic are achieved only when biomaterial pretreatment is carried out in accordance with the chapter *Sampling and Handling.*

13.2. Specificity

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

The PCR kit detects the DNA fragments of claimed pathogens. To confirm the analytical specificity the human DNA samples, as well as *Neisseria gonorrhoeae, Chlamidia trachomatis, Gardnerella vaginalis, Mycoplasma genitalium, Trichomonas vaginalis, Atopobium vaginae, Ureaplasma* sp., *Mycoplasma hominis, Ureaplasma parvum, Cytomegalovirus, Streptococcus agalactiae, HSV* I, *HSV* II, *EBV, Varicella-Zoster virus, Streptococcus pyogenes, Candida, Human papillomavirus* of low and unknown risk (genotypes 6, 11, 67, 70, 84, 81, 82, 62, 72, 73) were used. Nonspecific responses were absent.

The clinical specificity of **AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit was confirmed in laboratory clinical trials.

Reproducibility, repeatability and accuracy

Repeatability and reproducibility were determined by testing of the panel consisting of 14 samples. All the panel samples were prepared from the biological material containing DNA

of 14 *HPV* genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) with a concentration range from 3.2 to 4 lg copies/ml.

Table 6

Reproducibility

***HPV* genotype**	**Initial concentration value, lg copies/ml**	**Number of repeats**	**Average concentration value, lg copies/ml**	**Standard deviation (SD)**	**The coefficient of variation (CV), %**
16	3,2 - 4	40	3,55	0,04	1,19
18		40	3,94	0,07	1,71
31		40	3,73	0,09	2,55
33		40	3,68	0,13	3,44
35		40	3,45	0,15	4,30
39		40	3,66	0,12	3,32
45		40	3,65	0,06	1,58
51		40	3,50	0,14	4,09
52		40	3,64	0,11	2,96
56		40	3,78	0,13	3,36
58		40	3,79	0,11	2,86
59		40	3,46	0,09	2,46
66		40	3,88	0,09	2,35
68		40	3,60	0,13	3,53

Table 7

Repeatability

***HPV* genotype**	**Initial concentration value, lg copies/ml**	**Number of repeats**	**Average concentration value, lg copies/ml**	**Standard deviation (SD)**	**The coefficient of variation (CV), %**
16	3,2 - 4	40	3,56	0,04	1,18
18		40	3,91	0,06	1,50
31		40	3,78	0,05	1,19
33		40	3,57	0,03	0,95
35		40	3,33	0,07	2,18
39		40	3,56	0,08	2,19
45		40	3,61	0,03	0,84
51		40	3,37	0,04	1,09
52		40	3,58	0,10	2,76
56		40	3,68	0,08	2,13
58		40	3,70	0,08	2,07
59		40	3,41	0,08	2,40
66		40	3,81	0,06	1,61
68		40	3,51	0,07	2,12

The accuracy was determined by testing the quality control samples with the concentration of at least $5x10^4$ copies/ml.

Table 8

Accuracy

HPV genotype	Number of repeats	Average concentration value, lg copies/ml	Defined value lg copies/ml	Bias (B), %
16	40	3,55	3,72	4,63
18	40	3,94	3,68	6,62
31	40	3,73	3,67	1,63
33	40	3,68	3,66	0,59
35	40	3,45	3,78	9,49
39	40	3,66	3,77	3,01
45	40	3,65	3,71	1,80
51	40	3,50	3,62	3,60
52	40	3,64	3,72	2,37
56	40	3,78	3,76	0,33
58	40	3,79	3,81	0,48
59	40	3,46	3,69	6,68
66	40	3,88	3,61	5,37
68	40	3,60	3,81	5,60

Diagnostic characteristics

Diagnostic characteristics of **AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit were determined according to international requirements for validation of new tests for *HPV* DNA detection.

Diagnostic sensitivity of *HPV* test for CIN2+ detection should be not at least 90% of sensitivity of Hybrid Capture 2 method (HC2) (Digene hc2 High-Risk *HPV* DNA Test) according to international requirements for validation of new tests for *HPV* DNA. This means that relative sensitivity is at least 90% and the samples should be hystologically confirmed (CIN2 as a minimum). At least 60 samples should be tested using two *HPV* tests.

Diagnostic specificity of *HPV* test for CIN2+ detection should be not at least 98% of specificity of Hybrid Capture 2 method (HC2) (Digene hc2 High-Risk *HPV* DNA Test) according to international requirements for validation of new tests for *HPV* DNA. The sampling should include of at least 800 samples obtained from women over age 30 without cytologically/histologically confirmed CIN2.

The 888 samples (scrapings of membrane mucosa of cervix uteri, endocervical scrapings) were studied to determine diagnostic sensitivity and specificity of the kit. The 74 of these samples are with histologically confirmed diagnosis of CIN2+ (26 – CIN2, 37 – CIN3, 4 – AIS/ADC, 7 – SCC) and the average age of female patients is 35 years old (from 20 to 65 years old). And 814 of all samples obtained from screening study are with cytologically/hystologically confirmed absence of CIN2. An average age of the women is

39 years old (from 30 to 65 years old). HC2 test (Digene *HPV* test) was used as reference method.

Moreover 184 samples of vaginal mucosal swabs obtained from screening study were studied. **AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit (**REF** R-V31-T-4x(RG,iQ,Mx)-CE) was used as reference method.

Table 9

The results of testing AmpliSens® *HPV* HCR screen-titre-FRT PCR kit in comparison with reference method

Samples type	The results of application of AmpliSens® *HPV* HCR screen-titre-FRT PCR kit		The results of application of reference method[3]	
			Positive	Negative
Scraping of membrane mucosa of cervix uteri, endocervical scrapings with hystologically confirmed moderate or severe dysplasia (CIN2+)	74 samples were investigated	**Positive**	70	3
		Negative	0	1
Scraping of membrane mucosa of cervix uteri, endocervical scrapings with normal cytology or mild dysplasia	814 samples were investigated	**Positive**	89	13
		Negative	11	701
Swabs of vaginal mucosa	184 samples were investigated	**Positive**	46	8
		Negative	0	130

Table 10

Diagnostic characteristics of AmpliSens® *HPV* HCR screen-titre-FRT PCR kit

Samples type	Diagnostic sensitivity[4], %	Diagnostic specificity[5], %
Scraping of membrane mucosa of cervix uteri, endocervical scraping	100	98
Swab of vaginal mucosa	100	94

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, Moscow, 2010.

[3] Digene hc2 High-Risk HPV DNA Test kit for scrapings of membrane mucosa of cervix uteri, endocervical scrapings) and AmpliSens® *HPV* HCR screen-titre-FRT PCR kit (**REF** R-V31-T-4x(RG,iQ,Mx)-CE) (for swabs of vaginal mucosa) were used as reference method .

[4)] Relative sensitivity in comparison with applied reference method (Digene HPV test).

[5)] Relative specificity in comparison with applied reference method (Digene HPV test).

2. Guidelines to **AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit for detection and quantitation of the DNA of *human papillomaviruses* (*HPV*) of high carcinogenic risk (HCR) in the biological material by 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 accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Total Quality Management System, each lot of **AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

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