CE

IVD

For Professional Use Only

AmpliSens® *HPV* HCR genotype-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *HPV* HCR genotype-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of high carcinogenic risk (HCR) *human papillomaviruses* (*HPV*) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 DNA in the clinical material (cervical and urethral scrapes) by using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

The test is based on simultaneous PCR (multiplex-PCR) and real-time detection of three *HPV* types and β-globin gene DNA, used as internal control, in one tube. The analysis of 12 *HPV* types is carried out in four tubes. Each *HPV* type is registered on its own channel that allows not only to detect, but also to differentiate the virus genotype. DNA target selected as internal control is a fragment of human genome and must always be presented in sample (cervical swab) in sufficient amount that is equal to the amount of cells in the smear (10^3–10^5 of genomes). Therefore, endogenous internal control allows not only control stages of PCR (DNA isolation and PCR performance) but also evaluation of material obtaining and storage adequacy. If epithelial swab is obtained with mistakes (number of epithelial cells is insufficient), amplification signal of β-globin gene will be lowered.

3. CONTENT

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

AmpliSens® *HPV* HCR genotype-FRT PCR kit variant FRT **REF** R-V25(RG,iQ,Mx)-CE.

AmpliSens® *HPV* HCR genotype-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *HPV* 16/18/31	colorless clear liquid	0.08	6 blue cap tubes
PCR-mix-1-FRT *HPV* 39/45/59	colorless clear liquid	0.08	6 pink cap tubes
PCR-mix-1-FRT *HPV* 33/35/56	colorless clear liquid	0.08	6 green cap tubes
PCR-mix-1-FRT *HPV* 51/52/58	colorless clear liquid	0.08	6 orange cap tubes
PCR-buffer-FRT	colorless clear liquid	1.1	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	2 tubes
Positive Control DNA *HPV* types 16, 18, 31 and human DNA (C+$_{HPV\ 16,18,31}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 39, 45, 59 and human DNA (C+$_{HPV\ 39,45,59}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 33, 35, 56 and human DNA (C+$_{HPV\ 33,35,56}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 51, 52, 58 and human DNA (C+$_{HPV\ 51,52,58}$)	colorless clear liquid	0.06	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube

* must be used in isolation procedure as Negative control of Extraction

AmpliSens® *HPV* HCR genotype-FRT PCR kit is intended for 108 tests (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile DNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia) Instrument; iQ5 (BioRad, USA) Instrument; Mx3000P (Stratagene, USA) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) 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 barriers 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 detection.
- 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 one directional 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 samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

Clinical material:

For women: samples of epithelium are obtained by the same method as used for cytological analysis:

The first method – used kit for sampling consist of one/two cervical cytological brushes and 2 ml volume tube with 0.5 ml of Transport media with mucolytic agent.

Place cervical epithelial swab (endocervix), obtained with the first cervical cytological brush, and/or superficial cervical swab (ectocervix), obtained with the second cervical cytological brush, into the tube with transportation media. The working part of cytological brushes is to be broken off and left in the tube with transport media.

The second method - used kit for sampling, made by "Digene" (USA), consist of cervical cytological brush and a tube with 1.0 ml of transport media "Digene".

Place cervical epithelial swab (endocervix), obtained with cervical cytological brush, into the tube with transportation media "Digene".

The third method - used kit for sampling, consist of combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and 5 ml volume tube with 2.0 ml of Transport media with mucolytic agent.

Place cervical epithelial swab (endocervix) and superficial cervical swab (ectocervix) into the tube with transportation media. Working part of the probe is to be broken off and left in the tube with transport media.

The fourth method — used kit for sampling, consist of combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and jar with transport-fixation media made by "CytoScreen" (Italy) or "PreservCyt" (USA) for fluid cytology.

Place cervical epithelial swab (endocervix) and superficial cervical swab (exocervix) into the tube with transport-fixation media. Working part of the probe is to be broken off and left in the tube with transport media.

For men: Place the urethral epithelial swab obtained by universal probe, into the 2.0 ml volume tube with 0.5 ml 0.5 ml of Transport media with mucolytic agent.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR genotype-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 (see table 1):

Table 1

Material	DNA extraction kit	REF
Clinical material obtained by the 1st, 2nd, or 3rd method	DNA-sorb-B	K1-2-100-CE
	DNA-sorb-AM	K1-7-100-CE
Biopsy material from mucosa	DNA-sorb-C	K1-6-50-CE

Carry out the DNA extraction according to the manufacturer's instructions.

Note, that neither positive control, nor internal control are used for DNA extraction.

8.2. Preparing the PCR

Total reaction volume is **13 µl**, the volume of DNA sample is **5 µl**.

1. Prepare required number of tubes (0,1ml) (for 0,2-ml tubes with Rotor-Gene™ 6000 add 1 drop of mineral oil to each tube).
2. Add the whole volume of **polymerase (TaqF)** (60 µl) into the tube with **PCR-buffer-FRT** (1100 µl). Carefully vortex the tube**.** This mix is intended for 54 samples, including controls, and is stable for 3 months at +4°C.
3. Add **90 µl** of **polymerase (TaqF)** and **PCR-buffer-FRT** mix into each of the four **PCR-mix-1** tubes and carefully vortex the tubes (sufficient for genotyping of 16 clinical samples, including control samples reactions).
4. If it is necessary to test less than 16 samples prepare for each PCR-mix-1 one new tube and add for each sample **3.5*(N+2) µl** of **PCR-mix-1**, **4.5*(N+2)** of **polymerase (TaqF)** and **PCR-buffer-FRT mix**.

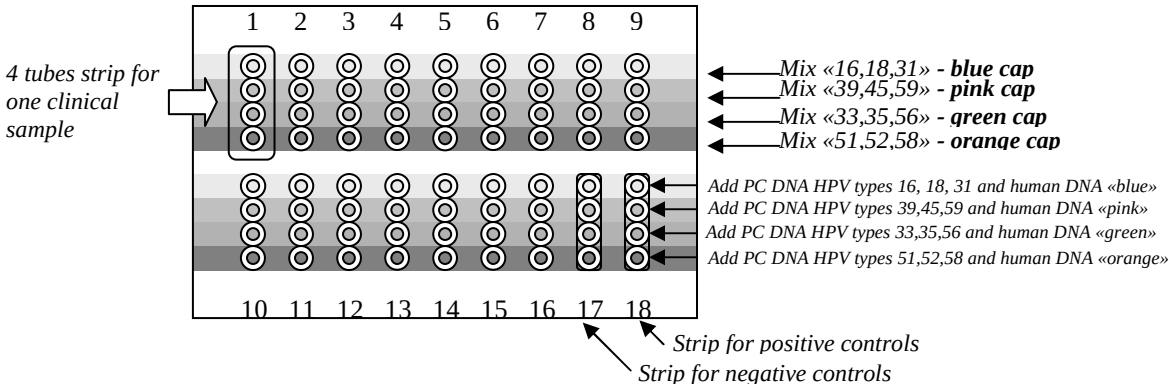
For example, for 8 clinical samples and 2 controls (Positive and Negative) prepare 35 µl of every PCR-mix-1 (3.5[8+2]) and add 45 µl of polymerase (TaqF) and PCR-buffer-FRT mix.*

The reaction mix (containing PCR-mix-1, polymerase (TaqF) and PCR-buffer-FRT) should be used within 2 hours.

5. Add **8.0 µl** of **Reaction Mix** into each tube. Dispense Reagents and Samples as shown below (every sample has to be tested in 4 tubes): Add to the first strip tube 8.0 µl of mix "16,18,31" (blue cap), to the second strip tube 8.0 µl of mix "39,45,59" (pink cap), to the third strip tube 8.0 µl of mix "33,35,56" (green cap), to the fourth tube 8.0 µl of mix

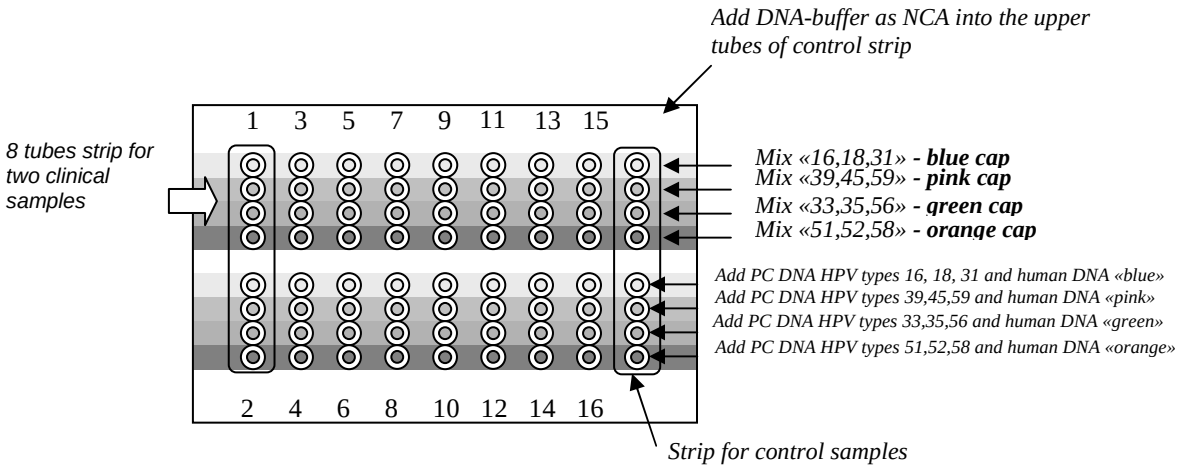
"51,52,58" (orange cap). When using 8-tubes strip, the tubes 5, 6, 7, 8 are prepared in the same way.

For Rotor-Gene 3000/6000:



Place strips as shown on the picture above. For 16 samples 18 strips are needed (for N samples – N+2 strips are needed).

For iQ5 and Mx3000P:



Place strips as shown on the picture above. For 16 samples 9 strips are needed (for N samples – N/2+1 strips are needed).

Be careful not to change the reaction mixes order in strips.

Add **5 µl** of **extracted DNA** sample into 4 tubes with different reaction mixes.

6. Carry out **control amplification reactions:**

NCA - Add **5 µl** of **DNA-buffer** into 4 tubes with different reaction mixes.

(C+$_{HPV\ 16,18,31}$) add **5 µl** of **Positive Control DNA *HPV* types 16, 18, 31 and human DNA** into the tube with "16,18,31" reaction mix;

(C+$_{HPV\ 39,45,59}$) add **5 µl** of **Positive Control DNA *HPV* types 39, 45, 59 and human DNA** into the tube with "39,45,59" reaction mix;

(C+$_{HPV\ 33,35,56}$) add **5 µl** of **Positive Control DNA *HPV* types 33, 35, 56 and human DNA**

into the tube with “33,35,56” reaction mix;

(C+$_{HPV\ 51,52,58}$) add **5 µl** of **Positive Control DNA *HPV* types 51, 52, 58 and human DNA** into the tube with “51,52,58” reaction mix;

8.3. Amplification

Program the Real-time instrument according to manufacturer’s manual and Guidelines [2].

8.3.1. For Rotor-Gene run one of the following amplification programs (see tables 2 and 3).

Table 2

AmpliSens-1 RG amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Repeats***
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 sec		

Table 3

RG amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Repeats***
Hold	95	15 min	–	1
Cycling	95	15 sec	–	45
	60	30 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	

8.3.2. For iQ iCycler, iQ run one of the following amplification programs (see tables 4 and 5).

Table 4

AmpliSens-1 iQ amplification program

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Repeats***
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sce	FAM, JOE/HEX, ROX, Cy5	
	72 °C	15 sec		

Table 5

iQ amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
Cycle 1	95	15 min	–	1
Cycle 2	95	15 sec	–	45
	60	50 sec	FAM, JOE/HEX, ROX, Cy5	

8.3.3 For Mx3000P and Mx3005P run one of the following amplification programs (see tables 6 and 7).

Table 6

AmpliSens-1 Mx amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
Segment 1	95	15 min	–	1
Segment 2 (Cycling)	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Segment 3 (Cycling)	95	5 sec	–	40
	60	30 sec	FAM, JOE, ROX, Cy5	
	72	15 sec	–	

Table 7

Mx amplification program

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Repeats*
Segment 1	95	15 min	–	1
Segment 2	95	20 sec	–	45
	60	60 sec	по Cy5, FAM, HEX, ROX	

9. DATA ANALYSIS

For data analysis refer to Guidelines [2].

Signal in the tube on the channel is considered to be positive, if the corresponding fluorescence accumulation curve cross threshold line. The signal is characterized by threshold cycle — that is, the cycle corresponding to the cross point of fluorescence curve and threshold line.

The result is ***valid*** if:

- Negative controls contain no signal on all channels (FAM/Green, JOE/Yellow/HEX/TET, ROX/Orange, Cy5/Red);
- All 12 *HPV* HCR types are detected in positive control samples.

If the reaction is invalid, all obtained data are considered to be invalid, and the reaction must be repeated.

The sample result of *HPV* DNA detection and genotyping is considered to be:

- *invalid,* if no positive signal is detected on any channel in any strip tube, including IC channel (Cy5/Red). (For Rotor-Gene only: a sample is considered to be invalid if only the IC signal is detected and the Ct value for this channel exceeds 35 if running the RG amplification program or 30 if running the Amplisens-1RG program).
- *negative*, if IC signal is present in all four tubes (Cy5/Red channel) and positive signals are not detected on any other channel (FAM/Green, JOE/Yellow, ROX/Orange).
- *positive,* in all other cases. IC signal can be absent in positive samples.

For Rotor-Gene only: the sample is considered to be *weak*, if IC signal (Cy5/Red channel) is present in all strip tubes and Ct value is **less than 35** if running RG amplification program or 30 if running Amplisens-1RG program and there is a positive signal in any other channel that exceed 35 if running the RG amplification program or 30 if running Amplisens-1 RG program (an *equivocal* result for this *HPV* type).

For this sample PCR run has to be repeated. If in the second run the result is *positive*, the sample is considered to be *positive*. If in the second run the result is *weak* or *negative*, the sample is considered to be *negative.*

The absence of the IC signal (Cy5/Red channel) in a strip tube is acceptable if signal/signals in FAM/Green, JOE/Yellow, and ROX/Orange channels is/are detected and Ct values do not exceed 35 if running the RG amplification program or 30 if running the AmpliSens-1 RG program.

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If for Negative Controls (C–, NCA) any Ct value appears on any channel, 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 tests, and to take measures to detect and eliminate the source of contamination.
2. If in positive control samples not all 12 *HPV* HCR types are detected, it can suggest incorrect programming of the temperature profile of the instrument, incorrect configuration of the PCR reaction, or storage conditions of the kit components has not complied with the manufacturer's instruction, or the reagent kit has expired. Accurate programming of the

instrument (see 8.3), storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.

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

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* HCR genotype-FRT PCR kit** (except for PCR-mix-1-FRT *HPV* 16/18/31, PCR-mix-1-FRT *HPV* 39/45/59, PCR-mix-1-FRT *HPV* 33/35/56, PCR-mix-1-FRT *HPV* 51/52/58, polymerase (TaqF)) should be stored at 2–8 °C. All components of the **AmpliSens® *HPV* HCR genotype-FRT** PCR kit are to be stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *HPV* 16/18/31, PCR-mix-1-FRT *HPV* 39/45/59, PCR-mix-1-FRT *HPV* 33/35/56, PCR-mix-1-FRT *HPV* 51/52/58 and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FRT *HPV* 16/18/31, PCR-mix-1-FRT *HPV* 39/45/59, PCR-mix-1-FRT *HPV* 33/35/56, and PCR-mix-1-FRT *HPV* 51/52/58 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *HPV* HCR genotype-FRT PCR kit** is no less than $1x10^3$ genome equivalents per 1 ml of sample for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 types.

The claimed analytical performance characteristics of **AmpliSens® *HPV* HCR genotype-FRT** PCR kit are guaranteed only when additional reagent kits DNA-sorb-B, DNA-sorb-AM, DNA-sorb-C, or DNA-sorb-D (manufactured by FBIS CRIE) are used.

13.2. Specificity

Specificity of **AmpliSens® *HPV* HCR genotype-FRT** PCR kit is assured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

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

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics” developed by Federal State Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines to **AmpliSens® *HPV* HCR genotype-FRT** PCR kit for qualitative detection and differentiation of high carcinogenic risk (HCR) *human papillomaviruses* (*HPV*) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 DNA in the clinical material (cervical and urethral scrapes) by using 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 **AmpliSens® *HPV* HCR genotype-FRT** PCR kit has been 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	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
20.01.11	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-FRT *HPV* 16/18/31, PCR-mix-1-FRT *HPV* 39/45/59, PCR-mix-1-FRT *HPV* 33/35/56 and PCR-mix-1-FRT *HPV* 51/52/58 are to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Through the text	For Positive Controls Positive Control DNA *HPV* types 16, 18, 31 and human DNA, Positive Control DNA *HPV* types 39, 45, 59 and human DNA, Positive Control DNA *HPV* types 33, 35, 56 and human DNA, Positive Control DNA *HPV* types 51, 52, 58 and human DNA abbreviations $C+_{16,18,31}$, $C+_{39,45,59}$, $C+_{33,35,56}$, $C+_{51,52,58}$ were added
		Writing of causative agent *HPV* is changed to italic
22.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
12.07.12 LA	9. Data analysis	Ct values were changed throughout the text
21.12.12 LA	3. Content 8. Protocol	Short designations of the positive controls of amplification were corrected: $C+_{HPV\ 16,18,31}$, $C+_{HPV\ 39,45,59}$, $C+_{HPV\ 33,35,56}$, $C+_{HPV\ 51,52,58}$ instead of $C+_{16,18,31}$, $C+_{39,45,59}$, $C+_{33,35,56}$, $C+_{51,52,58}$, respectively