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IVD

For Professional Use Only

AmpliSens® *HPV* HCR genotype-EPh PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *HPV* HCR genotype-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of high carcinogenic risk (HCR) *Human Papillomavirus* (*HPV*) types 16, 31, 33, 35, 18, 39, 45, 59 and 52, 56, 58, 66 in the biological material (cervical or urethral swabs) using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

Human Papillomavirus of high carcinogenic risk detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *HPV* HCR primers. After PCR, the amplified product is detected in agarose gel. AmpliSens® *HPV* HCR genotype-EPh PCR kit is based on simultaneous amplification in one tube (multiplex-PCR) of four types of *Human Papillomavirus* DNA and β-globin gene, which is used as an endogenous Internal Control. PCR test for detection of DNA of twelve *HPV* types is performed in three tubes. Since all amplified products differ in length, the genotype of the virus can be identified. The DNA target selected as an endogenous Internal Control is a human genome fragment. It must be present in the sample in a sufficient quantity equivalent to that of cells in the sample (not less than 10^3–10^5 genomes). If the number of epithelial cells in the sample is insufficient or the amount of mucus is too high, the band corresponding to the Internal Control will be absent in agarose gel.

AmpliSens® *HPV* HCR genotype-EPh PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENTS

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

AmpliSens® *HPV* HCR genotype-EPh PCR kit variant 50 F, **REF** V25-50-F-CE.

AmpliSens® *HPV* HCR genotype-EPh PCR kit variant 50 F includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1 *HPV* 16/35	colorless clear liquid	0.3	1 tube
PCR-mix-1 *HPV* 18/59	colorless clear liquid	0.3	1 tube
PCR-mix-1 *HPV* 52/66	colorless clear liquid	0.3	1 tube
2.5x PCR-buffer red	red clear liquid	0.6	3 tubes
Polymerase (TaqF)	colorless clear liquid	0.09	1 tube
Mineral oil for PCR	colorless viscous liquid	8.0	1 vial
Positive Control Glob ($C+_{Glob}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	3 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see DNA-sorb-AM **REF** K1-12-50-CE Protocol).

The Panel of Positive Control samples of *HPV* HCR DNA includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
Positive Control *HPV* 16 ($C+_{HPV\ 16}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 31 ($C+_{HPV\ 31}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 33 ($C+_{HPV\ 33}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 35 ($C+_{HPV\ 35}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 18 ($C+_{HPV\ 18}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 45 ($C+_{HPV\ 45}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 39 ($C+_{HPV\ 39}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 59 ($C+_{HPV\ 59}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 52 ($C+_{HPV\ 52}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 56 ($C+_{HPV\ 56}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 58 ($C+_{HPV\ 58}$)	colorless clear liquid	0.15	1 tube
Positive Control *HPV* 66 ($C+_{HPV\ 66}$)	colorless clear liquid	0.15	1 tube

AmpliSens® *HPV* HCR genotype-EPh PCR kit variant 50 F is intended for 165 reactions (55 tests), including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Detection agarose kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile DNase-free pipette tips with filters (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with a rotor for 2-ml reaction tubes (RCF max. 16,000 x g).
- PCR box.
- Vacuum aspirator with flask for removing supernatant.
- Tube racks.
- 1.5-ml sterile polypropylene tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer at minus 24 to minus 16 °C.
- Reservoir for used tips.
- Permanent pen for labeling.
- Thermostatic bath or dry block for tubes with controlled temperature for 25–100 °C.
- Programmable thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen Scientific, USA)).

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with filters and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use protective gloves, laboratory coats, 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 samples 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 sample or reagent spills with 0.5% sodium hypochlorite solutions 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.
- Material Safety Data Sheets (MSDS) 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 to 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 [2]. It is recommended that this handbook is read before starting work.

AmpliSens® *HPV* HCR genotype-EPh PCR kit is intended for analysis of DNA extracted with DNA extraction kits from cervical or urethral swabs.

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 for Swabs **REF** 956-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 should 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 transportation medium.

Method 3. The sampling kit consists of a combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix and a 5-ml tube with 2.0 ml of Transport Transport Medium for Swabs **REF** 956-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 for Swabs **REF** 956-CE.

Sample storage conditions:

- at temperature from 18 to 25 °C – no more than 5 days;
- at temperature from 2 to 8 °C – no more than 20 days;
- at 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 stored at room temperature throughout the year.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR genotype-EPh 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** K1-12-50-CE (for biological material obtained by methods 1, 2, and 3).
- DNA-sorb-C **REF** K1-6-50-CE (for mucous biopsy material).

Extract the DNA according to the manufacturer's protocol.

In case of extracting with DNA-sorb-AM reagent kit, take into account the following improvements:

- Do not add the Internal Control complex (ICc) to the tubes (
- In case of testing more than 50 samples, it is acceptable to mix the reagents by the 2nd method, but:
  - Do not add the Internal Control complex (ICc) (1 ml of Universal Sorbent per 15 ml of Lysis solution
  - To each tube add 320 µl of Lysis Solution and Universal Sorbent mixture
  - The purified DNA could be stored at the temperature range from minus 24 to minus 16 °C for 1 year.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the reaction mixture with **PCR-mix-1 *HPV* 16/35** for N reactions:

   5∗(N+1) µl of PCR-mix-1 *HPV* 16/35

   10∗(N+1) µl of 2.5x PCR-buffer red

   0.5∗(N+1) µl of polymerase (TaqF)

When calculating the reaction mixture volume, additional reactions should be included: six controls (one negative and five positive) and one extra reaction.

2. Mix by vortexing the tube with the reaction mixture. Add 15 µl of the reaction mixture to PCR tubes.
3. Add above 1 drop of **mineral oil for PCR**.
4. Prepare reaction mixtures with **PCR-mix-1 *HPV* 18/59** and **PCR-mix-1 *HPV* 52/66** as described above.
5. Add reaction mixture **16–35** to the blue tubes, reaction mixture **18–59** to the pink tubes, and reaction mixture **52–66** to the green tubes.
6. Arrange tubes in three rows in a PCR tube rack.
7. Using tips with filter, add **10 µl DNA samples** obtained from biological or control samples. DNA of the same biological sample should be added to the corresponding tubes of all three rows.
8. Carry out the control amplification reactions:

NCA	- Instead of sample DNA add 10 µl of **TE-buffer** to the tube for Negative Control of Amplification (NCA).
C+$_{Glob}$	- Add 10 µl of **Positive Control Glob (C+$_{Glob}$)** to the tube for Positive Control of human DNA
C+$_{HPV\ 16}$, C+$_{HPV\ 31}$, C+$_{HPV\ 33}$, C+$_{HPV\ 35}$ (reaction mixture 16-35)	- Add 10 µl of **Positive Control HPV 16 (**C+$_{HPV\ 16}$**),** 10 µl of **Positive Control *HPV* 31 (**C+$_{HPV\ 31}$**),** 10 µl of **Positive Control *HPV* 33 (**C+$_{HPV\ 33}$**),** 10 µl of **Positive Control *HPV* 35 (**C+$_{HPV\ 35}$**)** to four blue tubes for Positive Controls of *HPV*.
C+$_{HPV\ 18}$, C+$_{HPV\ 39}$, C+$_{HPV\ 45}$, C+$_{HPV\ 59}$ (reaction mixture 18-59)	- Add 10 µl of **Positive Control *HPV* 18 (**C+$_{HPV\ 18}$**),** 10 µl of **Positive Control *HPV* 39 (**C+$_{HPV\ 39}$**),** 10 µl of **Positive Control *HPV* 45 (**C+$_{HPV\ 45}$**),** 10 µl of **Positive Control *HPV* 59 (**C+$_{HPV\ 59}$**)** to four pink tubes for Positive Controls of *HPV*.
C+$_{HPV\ 52}$, C+$_{HPV\ 56}$, C+$_{HPV\ 58}$, C+$_{HPV\ 66}$ (reaction mixture 52-66)	- Add 10 µl of **Positive Control *HPV* 52 (**C+$_{HPV\ 52}$**),** 10 µl of **Positive Control *HPV* 56 (**C+$_{HPV\ 56}$**),** 10 µl of **Positive Control *HPV* 58 (**C+$_{HPV\ 58}$**),** 10 µl of **Positive Control *HPV* 66 (**C+$_{HPV\ 66}$**)** to four green tubes for Positive Controls of *HPV*.

8.2.2 Amplification

1. Run the following program in the thermocycler (see Table 1). When the temperature reaches 95 °C (pause mode), insert tubes into the cells of the thermocycler and press the

button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.

Table 1

Programming thermocyclers for amplification of DNA of *HPV* HCR types 16, 31, 33, 35; 18, 39, 45, 59 and 52, 56, 58, 66

	Thermocyclers with active temperature adjustment:			Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2700 (Applied Biosystems), Terzik (DNA-Techology)			**PTC-100 (MJ Research), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**		
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles
1	**95 °C**	**15 min**	1	**95 °C**	**15 min**	1
2	**95 °C**	30 s	42	**95 °C**	30 s	42
	63 °C	30 s		**63 °C**	40 s	
	72 °C	40 s		**72 °C**	50 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**10 °C**	storage		**10 °C**	storage	

2. Amplification in thermocyclers with block temperature adjustment lasts for 2 h 30 min; in thermocyclers with active temperature adjustment, for 1 h 50 min.
3. After the reaction is finished, PCR tubes must be collected and transferred to the room for PCR product analysis.

The amplification products are analyzed by separation of DNA fragments in agarose gel.

The amplified samples can be stored at room temperature for 16 h and at 2–8 °C for 1 week and long time at temperatures from minus 24 to minus 16 °C (be sure to warm the samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

It is recommended to use the following detection agarose kit:

- EPh variant genotype-300, **REF** K6-300-CE.

Use EPh kit according to the manufacturer's protocol, taking into account the following additions and improvements:

- Prepare **control samples of electrophoresis** in detection area:

$CPh+_{16-35}$ - mix in one clean tube the content of four blue tubes containing amplified products of control panel samples $C+_{HPV\ 16}$, $C+_{HPV\ 31}$, $C+_{HPV\ 33}$, $C+_{HPV\ 35}$

$CPh+_{18-59}$ - mix in one clean tube the content of four pink tubes containing amplified products of control panel samples $C+_{HPV\ 18}$, $C+_{HPV\ 39}$, $C+_{HPV\ 45}$, $C+_{HPV\ 59}$

$CPh+_{52-66}$ - mix in one clean tube the content of four green tubes containing amplified products of control panel samples $C+_{HPV\ 52}$, $C+_{HPV\ 56}$, $C+_{HPV\ 58}$, $C+_{HPV\ 66}$

Put on a protective mask or use a glass filter while watching and photographing the gel.

The tube with PCR-mix-1 *HPV* 16/35 contains the primers for amplifying *HPV* types 16, 31, 33, 35 DNA; the tube with PCR-mix-1 *HPV* 18/59 contains primers for amplifying *HPV* types 18, 39, 45, 59 DNA; and the tube with PCR-mix-1 *HPV* 52/66 contains primers for amplifying *HPV* types 52, 56, 58, 66 DNA. Each tube contains primers for amplifying the human genome (β-globin gene) DNA fragment.

Table 2

The length of specific amplified DNA fragments

PCR-mix-1 ~~R~~ *HPV* 16/35			**PCR-mix-1 ~~R~~ *HPV* 18/59**			**PCR-mix-1 ~~R~~ *HPV* 52/66**		
Virus genotype	**The length of specific amplified DNA fragment**	**The length of internal control amplified fragment**	**Virus genotype**	**The length of specific amplified DNA fragment**	**The length of internal control amplified fragment**	**Virus genotype**	**The length of specific amplified DNA fragment**	**The length of internal control amplified fragment**
HPV 16	325 bp	723 bp	*HPV* 18	425 bp	723 bp	*HPV* 52	360 bp	723 bp
HPV 31	520 bp		*HPV* 45	475 bp		*HPV* 56	325 bp	
HPV 33	227 bp		*HPV* 39	340 bp		*HPV* 58	240 bp	
HPV 35	280 bp		*HPV* 59	395 bp		*HPV* 66	304 bp	

9.1. Interpretation of results

Table 3

Results for controls

Control	Controlled step	Specific bands in agarose gel				Interpretation
		723 bp	**325 bp, 520 bp, 227 bp, 280 bp**	**425 bp, 475 bp, 340 bp, 395 bp**	**360 bp, 325 bp, 240 bp, 304 bp**	
C-	DNA extraction	No	No	No	No	OK
NCA	PCR	No	No	No	No	OK
$C+_{Glob}$	PCR	Yes	No	No	No	OK
$CPh+_{16-35}$	Electrophoresis	No	Yes	No	No	OK
$CPh+_{18-59}$	Electrophoresis	No	No	Yes	No	OK
$CPh+_{52-66}$	Electrophoresis	No	No	No	Yes	OK

- The sample is considered to be **positive** if one or more specific bands are present in agarose gel:

  – 325, 520, 227 or 280 bp – if amplified with reaction mixture 16-35 (blue tubes);

  – 425, 475, 340 or 455 bp – if amplified with reaction mixture 18-59 (pink tubes);

  – 360, 325, 240 or 304 bp – if amplified with reaction mixture 52-66 (green tubes);

  – and the band of Internal Control (**723 bp) –** in all tubes regardless of their color.

- The sample is considered to be negative if only the 723-bp Internal Control band is present.

In addition to the specific bands, fuzzy bands corresponding to primer dimers may appear in lanes below the 100-bp level.

Line the amplified bands of biological samples with corresponding bands of CPh+ in order to identify virus genotype (Appendix 1).

10. TROUBLESHOOTING

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

1. If results of analysis of control points (NCA, CPh+) do not correspond to those listed above (Table 3).
2. If the Internal Control band (723 bp) is absent in one of three lanes corresponding to the sample, the result of sample analysis is invalid. PCR amplification should be repeated.
3. If the Internal Control band (723 bp) is not observed in all three lanes corresponding to the sample, the result of sample analysis is invalid and analysis of this sample must be repeated from the DNA extraction stage. If a similar result is obtained for the second time, sampling should be repeated.
4. The appearance of nonspecific bands of different molecular weight in lanes may be caused by the lack of “hot start” or an inappropriate temperature regime in the thermocycler. In this case, the results of analysis are invalid.
5. The appearance of specific bands in the lanes corresponding to negative controls (NCA)

suggests contamination of reagents or samples. In such cases, the results of analysis are considered to be invalid. Analysis of all samples must be repeated and measures to detect and eliminate the source of contamination must be taken.

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

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of **AmpliSens® *HPV* HCR genotype-EPh** PCR kit (except for polymerase (TaqF) are to be stored at 2–8 ºC when not in use. All components of **AmpliSens® *HPV* HCR genotype-EPh** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) is to be stored at temperature from minus 24 to minus 16 ºC when not in use.

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml[1)]	Detection kit
Cervical canal epithelial scrape (endocervical) and cervix epithelial scrape	DNA-sorb-AM (REF K1-12-100-CE)	PCR kit variant 100 R	$2,5x10^4$	EPh (REF K6-300-CE)
Urethral epithelial scrape				

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

13.2. Specificity

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

[1)] Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

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

14. REFERENCES

1. Wieland U, Pfister H. Molecular diagnosis of persistent human papilloma virus infections.Intervirology. 1996; 39(3):145-57.
2. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2010.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *HPV* HCR genotype-EPh** 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	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**$C+_h$**	Positive Control DNA human
EC REP	Authorised representative in the European Community	**IC**	Internal control
		$CPh+_{16-35}$ **$CPh+_{18-59}$** **$CPh+_{52-66}$**	Control samples of electrophoresis

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
09.12.10	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 open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
22.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
10.02.14 SA	Text	“Clinical material” was changed to “biological material”
		“Isolation” was changed to “extraction”
		“Aerosol barriers” were changed to “filters”
		Positive Control DNA human was changed to Positive Control Glob
		DNA-buffer was changed to TE-buffer Positive Control DNA human ($C+_{h}$) was changed to Positive Control Glob ($C+_{Glob}$) Positive Control DNA *HPV* type 16 ($C+_{HPV\ 16}$) was changed to Positive Control *HPV* 16 ($C+_{HPV\ 16}$) Positive Control DNA *HPV* type 31 ($C+_{HPV\ 31}$) was changed to Positive Control *HPV* 31 ($C+_{HPV\ 31}$) Positive Control DNA *HPV* type 33 ($C+_{HPV\ 33}$) was changed to Positive Control *HPV* 33 ($C+_{HPV\ 33}$) Positive Control DNA *HPV* type 35 ($C+_{HPV\ 35}$) was changed to Positive Control *HPV* 35 ($C+_{HPV\ 35}$) Positive Control DNA *HPV* type 18 ($C+_{HPV\ 18}$) was changed to Positive Control *HPV* 18 ($C+_{HPV\ 18}$) Positive Control DNA *HPV* type 45 ($C+_{HPV\ 45}$) was changed to Positive Control *HPV* 45 ($C+_{HPV\ 45}$) Positive Control DNA *HPV* type 39 ($C+_{HPV\ 39}$) was changed to Positive Control *HPV* 39 ($C+_{HPV\ 39}$) Positive Control DNA *HPV* type 59 ($C+_{HPV\ 59}$) was changed to Positive Control *HPV* 59 ($C+_{HPV\ 59}$) Positive Control DNA *HPV* type 52 ($C+_{HPV\ 52}$) was changed to Positive Control *HPV* 52 ($C+_{HPV\ 52}$) Positive Control DNA *HPV* type 56 ($C+_{HPV\ 56}$) was changed to Positive Control *HPV* 56 ($C+_{HPV\ 56}$) Positive Control DNA *HPV* type 58 ($C+_{HPV\ 58}$) was changed to Positive Control *HPV* 58 ($C+_{HPV\ 58}$) Positive Control DNA *HPV* type 66 ($C+_{HPV\ 66}$) was changed to Positive Control *HPV* 66 ($C+_{HPV\ 66}$)
	3. Content	The volume and quantity of TE-buffer was changed at 0.5 and 1 tube to 0.2 and 3 tubes
	4. Additional requirements	“Deep-freezer for ≤ –16 °C” was changed to “Deep-freezer at minus 24 to minus 16 °C”
	6. Sampling and handling	Sample storage conditions were added
	8. Protocol	The chapter was completed
	9. Data analysis	The chapter was completed
	10.	The chapter was corrected

	Troubleshooting	
	13. Specifications	Sensitivity was changed at “no less than $5x10^3$ GE/ml” to “no less than $2,5x10^4$ GE/ml” DNA-sorb-B and DNA-sorb-C reagents kit were deleted