CE

IVD

For Professional Use Only

AmpliSens® *HPV* HCR screen-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *HPV* HCR screen-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *human papillomavirus* (*HPV*) of high carcinogenic risk (HCR) types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 DNA in the biological material (urogenital 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

HPV HCR 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 screen-EPh** PCR kit is able to detect three main phylogenetic groups of *HPV*: A7, A6, and A9 that include the following types: 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70. These types are known for high transformation ability and they are responsible for more than 97% of severe cervical dysplasia and cervical cancer. **AmpliSens® *HPV* HCR screen-EPh** PCR kit contains the internal endogenous control (fragment of β-globine gene).

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

The test is based on simultaneous amplification (multiplex PCR) of E1-E2 gene fragments of three *HPV* groups and a fragment of β-globin gene which is used as an internal endogenous control. PCR test detecting fourteen HPV types of is run in a single tube. All amplified products have a similar length.

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

Nowadays epidemiologic studies show that most routine screening tests for dysplastic changes of cervix, vagina, and vulva as well as for evaluation of the risk of their development require detection of *clinically valuable* quantity of high-risk *HPV*. It is believed, that detection of a virus in quantity not exceeding the certain threshold value is clinically insignificant, due to the fact that 100% of such cases are associated with spontaneous recovery. On the contrary, the high virus load indicates dysplasia or the risk of its development. However, if it is not the prophylactic (screening) examination, but the controlled treatment, then even low virus

quantity can be an early marker of a relapse. Currently, the level of clinically significant virus quantity estimates 10^5 GE of HCR *HPV* per cervical swab when standard obtaining of biological material is provided.

Clinical trials with model samples demonstrated that PCR kit allows detection of the clinically significant virus quantity in the case of standard sampling of urogenital scraps into 0.5 ml of transport medium, subsequent DNA extraction, 100x dilution of obtained DNA samples and amplification.

Clinical trials on samples collected from both healthy patients and patients suffering from severe dysplasia and cervical cancer demonstrated increase of dysplasia detection specificity by 22.9% (from 61.7% without dilution to 84.6% with dilution) whereas the high level of severe dysplasia and cervical cancer diagnosis was maintained (98.9%).

Note that level of clinically significant virus quantity was not validated in men.

Therefore, AmpliSens® *HPV* HCR screen-EPh PCR kit can run two formats of high-risk HPV detection:

- ***HPV* HCR presence** (a sample is tested after DNA extraction).
- ***HPV* HCR clinically significant quantity** (a sample is tested after DNA extraction and dilution with TE-buffer). Note that standard sampling is necessary.

3. CONTENTS

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

AmpliSens® *HPV* HCR screen-EPh PCR kit variant 100 F **REF** V31-100F-CE.

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

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix -1 *HPV* HCR screen	colorless clear liquid	0.3	2 tubes
2.5x PCR-buffer blue	blue clear liquid	1.15	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 tube
Positive Control *HPV* 31, 39, 56 / Glob ($C+_{HPV\ 31, 39, 56 / Glob}$)	colorless clear liquid	0.2	1 tube
Positive Control Glob ($C+_{Glob}$)	colorless clear liquid	0.2	1 tube
TE-buffer*	colorless clear liquid	5.0	4 tubes
Negative Control (C-)**	colorless clear liquid	1.2	1 tube

* must be used in the amplification procedure as Negative Control of Amplification

** must be used in the extraction procedure as Negative Control of Extraction (see DNA-

sorb-AM, **REF** K1-12-100-CE, or DNA-sorb-B, **REF** K1-2-100-CE or DNA-sorb-C, **REF** K1-6-50-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (up to 200 µl).
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Terzik (DNA-Technology), GeneAmp PCR System 2700 (Applied Biosystems), MaxyGene (Axygen).
- Disposable polypropylene microtubes for PCR with 0.2- 0.5-ml capacity (for example, Axygen, USA)**.**
- Refrigerator for 2-8 °C.
- Deep-freezer with the temperature range from minus 24 to minus 16 °C.
- Reservoir 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 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 disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 [1]. It is recommended that this handbook is read before starting work.

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

- *Urogenital swabs*

Female: epithelium samples should be obtained as for cytological examination:

Method 1 - use the sampling kit which includes one/two cervical cytobrushes and the 2-ml tube with 0.5 ml of Transport Medium for Swabs **REF** 956-CE.

Endocervical epithelial scrape taken with the first cytobrush and/or exocervical epithelial scrape taken with the second cytobrush should be placed into the tube with a transport medium. Break the effective part of the cytobrush with the sample at the score mark and leave it in the tube.

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

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

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 for Swabs **REF** 956-CE.

Place endocervical and exocervical epithelial scrapes into the tube with transport media. 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 the 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 the urethral epithelial scrape by a universal probe and place it into the 2.0-ml tube with 0.5 ml of 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 screen-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

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

- DNA-sorb-AM, **REF** K1-12-100-CE (for biological material obtained by methods 1, 2, and 3).

Extract the DNA according to the manufacturer's protocol taking into account next improvement:

- Don't add Internal Control complex (ICc);

- DNA-sorb-B, **REF** K1-2-100-CE (for biological material obtained by methods 1, 2, and 3).
- DNA-sorb-C, **REF** K1-6-50-CE (is used for mucosa biopsy samples).

8.2. Preparing the PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare reaction mix for N reactions:

   5∗(N+1) µl of PCR-mix-1 *HPV* HCR screen

   10∗(N+1) µl of 2.5x PCR-buffer blue

   0.5∗(N+1) µl of polymerase (TaqF)

Calculate the reaction mixture volume taking into account three controls (one negative and two positive) and one extra reaction.

The prepared reaction mixture can be stored for up to 2 hours.

2. Vortex the tube with the reaction mixture. Add 15 µl of the reaction mixture into PCR tubes.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). Omit if a thermocycler with constant-temperature cover is used.
4. Using tips with filter add **10 µl** of **DNA samples** obtained from test or control samples at the DNA extraction stage, under oil or directly on the level of oil.(in case of *clinically significant quantity of HPV HCR format* use 100x dilution of obtained DNA samples).
5. Carry out the control amplification reactions:

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

$C+_{Glob}$ - Add 10 µl of **Positive Control DNA Glob ($C+_{Glob}$)** to the tube for Positive Control of human DNA Amplification.

$C+_{HPV\ 31,\ 39,\ 56}$ - Add 10 µl of **Positive Control *HPV* 31, 39, 56 / Glob ($C+_{HPV\ 31,\ 39,\ 56\ /\ Glob}$)** to the tube for Positive Control of HPV Amplification.

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.

The run takes approximately 2 h 30 min to complete in a thermocycler with block temperature adjustment or 1 h 50 min in a thermocycler with active temperature adjustment.

Table 1

Programming thermocyclers for DNA amplification of DNA of HPV HCR types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	Terzik (DNA-Technology)			**GeneAmp PCR System 2400 (Perkin Elmer), GeneAmp PCR System 2700 (Applied Biosystems)**			**T-personal (Biometra), PTC-100 (MJ Research)**		
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles
1	**95 °C**	900 s	1	**95 °C**	900 s	1	**95 °C**	900s	1
2	**95 °C**	10 s	42	**95 °C**	15 s	42	**95 °C**	30 s	42
	63 °C	20 s		**63 °C**	30 s		**63 °C**	40 s	
	72 °C	20 s		**72 °C**	30 s		**72 °C**	40 s	
3	**72 °C**	60 s	1	**72 °C**	60 s	1	**71 °C**	60 s	1

2. After the reaction is completed, the PCR tubes must be collected and sent to the room for PCR products analysis. Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C (make sure that the samples are warmed up to room temperature before running electrophoresis).

9. DATA ANALYSIS

It's recommended that the following detection agarose kit is used:

- EPh variant 200, **REF** K5-200-CE.

Use EPh kit according to the manufacturer's protocol.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%).

PCR-mix -1 HPV HCR screen includes primers for amplification of 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 types DNA fragments as well as the fragment of human genome DNA (β-globin gene).

The length of specific amplified DNA fragments is:

- Internal Control (fragment of β-globin gene) - 723 bp
- HPV types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 - from 267 to 325 bp

Use a protective mask or a glass filter when looking at the gel or taking photos

9.1. Results interpretation

Table 2

Results for controls

Control	Step for control	Specific bands in the agarose gel		Interpretation
		267-325 bp	723 bp	
C-	DNA extraction	No	No	OK
NCA	Amplification	No	No	OK
C+$_{Glob}$	Amplification	No	Yes	OK
C+$_{HPV\ 31,\ 39,\ 56}$	Amplification	Yes	Yes	OK

- The sample is considered positive for *HPV* HCR DNA if the band at the level from 267 to 325 bp is present in agarose gel regardless of the band of Internal Control (723 bp).
- The sample is considered negative for *HPV* HCR DNA if the only 723-bp band is present.
- Besides the specific products the fuzzy bands of primer dimers may appear in the lanes below the 100-bp level.

10. TROUBLESHOOTING

Analysis results are not obtained as per the following examples:

- If the results of the controls (C+$_{Glob}$, C+$_{HPV\ 31,\ 39,\ 56}$) do not match with the listed above (Table 2), then the appropriate step of the test should be repeated.
- If the Internal Control band (723 bp) is not observed in the lane of the test sample, it can indicate that the insufficient quantity of biological material was taken or mistakes in clinical processing, DNA extraction, or PCR conducting were made.
- If nonspecific bands are seen at different levels in the lanes, this may be caused by the lack of “hot start” or incorrect temperature profile of the thermocycler.
- If the specific bands appear in the lanes corresponding to the negative controls (NCA) it indicates contamination of the reagents or samples. In this case results of the analysis for all samples are considered invalid. The test must be repeated and measures to detect and eliminate the source of contamination should be taken.

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

11. TRANSPORTATION

AmpliSens® *HPV* HCR screen-EPh 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-EPh** PCR kit are to be stored at 2-8 ºC (except for polymerase TaqF) when not in use. All components of the PCR kit are to be stable until the labeled expiration date. 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** K5-200-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 screen-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.

The clinical specificity of **AmpliSens® *HPV* HCR screen-EPh** 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 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 screen-EPh** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

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

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	**C+**$_{HPV\ 31,\ 39,\ 56}$	Positive Control DNA *HPV* types 31, 39, 56 and DNA human
	Manufacturer	**C+**$_{Glob}$	Positive Control DNA human
	Date of manufacture	**NCA**	Negative control of amplification
EC REP	Authorised representative in the European Community	**C–**	Negative control of extraction

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
06.07.10	Content	«C+$_{HPV\ 31,\ 39,\ 56}$» was added for Positive Control DNA *HPV* types 31, 39, 56 and DNA human, «C+$_h$» - for Positive Control DNA human
25.12.10 KM	Through the text	Correcting the document template
		«Cervical or urethral scrapes» was changed to «urogenital swabs»
	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”
23.07.12 Ivl	Content	AmpliSens® *HPV* HCR screen-EPh PCR kit variant 50 F was deleted
	Footer	Catalogue number REF V31-50F-CE was deleted
10.02.14 GA	Text	“Clinical material” was changed to “biological material”
		Positive Control DNA human (C+$_h$) was changed to Positive Control Glob (C+$_{Glob}$)
		Positive Control DNA *HPV* types 31, 39, 56 and DNA human (C+$_{HPV\ 31,\ 39,\ 56}$) was changed to Positive Control *HPV* 31, 39, 56 / Glob (C+$_{HPV\ 31,\ 39,\ 56\ /\ Glob}$)
	4. Additional requirements	Instruments GeneAmp PCR System 2400T-personal (Biometra), PTC-100 (MJ Research) MaxyGene (Axygen) wad deleted The chapter was corrected in accordance to the template
	6. Sampling and handling	“Transport medium TSM” was changed to “Transport Medium for Swabs **REF** 956-CE” Sample storage conditions was added
	8. Protocol	The chapter was completed
	9. Data analysis	The chapter was completed
	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 Attention “This sensitivity is achieved in compliance with the above rules, recommended sample preparation of biomaterial and sample volume” was added Phrase about *HPV* types was deleted Specificity was rewritten with accordance to the template