ECOLI

eSens

CE

IVD

For Professional Use Only

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit

REF ES3080A

Instructions for Use

1 INTENDED USE

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of DNA of *human papillomaviruses* of high carcinogenic risk (HPV HR) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 in the clinical material (vaginal swab, epithelial scrape from the cervical mucous membrane (ectocervix and endocervix)) using real-time hybridization-fluorescence detection of amplified products.

PCR kit allows to separately identify the HPV DNA of genotypes 16, 18 and 45. HPV HR are the main etiological factor in the development of cervical cancer and the previous high grade dysplasia. HPV is detected in 95 % of all cervical cancer cases. The specified genotypes 16, 18 and 45 in conjunction are known to cause around 75% squamous cell carcinomas and 94% cervical adenocarcinoma cases. The PCR kit allows to determine the total number of HPV DNA of all 14 genotypes and also to separately identify the HPV DNA of 16, 18 and 45 genotypes.

It is known that development of cervical cancer is often associated with integration of viral DNA into the genome of the host cell. HPV of genotypes 16 and 18 integrate the most frequently, while the E1/E2 region is being broken and E6/E7 oncogene persists. The **eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit** has been developed to detect different genome section of HPV types 16, 18 and 45 in the different channels of the instrument. Detection of E6 region (in the absence of E1/E2 region) allows indirectly to judge about the possibility of virus integration into the human genome.

PCR kit is used in the clinical laboratory diagnostics for studying the biological material, taken from the persons suspected of papillomavirus infection without distinction of form and presence of disease manifestation.

The material for PCR is the DNA samples extracted from the test material with the use of nucleic acids extraction kits recommended by the manufacturer.

NOTE: The results of PCR analysis are to be taken into account in complex diagnostics of disease.

2 PRINCIPLE OF PCR DETECTION

HPV HR 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 amplification of DNA fragments of HPV genotypes and a DNA fragment of β-globin gene. DNA fragment of β-globin gene is used as an internal endogenous (IC) control. The use of an endogenous internal control makes it possible not only to monitor all test stages but also to assess the adequacy of sampling and storage of clinical material. An endogenous internal control is a human genome fragment. It must be always present in the sample in sufficient quantities equivalent to the number of cells in the sample (500 – 10^5 cells/reaction).

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Quantitative analysis of HPV HR 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). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of the concentration of the DNA target in test samples.

HPV DNA concentration is calculated as the relation between number of HPV copies and number of epithelial cells of human membrane mucosa.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So, the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

HPV HR genotype detection (including differentiation of genotypes 16, 18 and 45) is carried out for one sample in one tube in which 14 HPV HCR genotypes and IC Glob are detected. The results of amplification of different genotypes of HPV HR DNA and IC Glob DNA for each PCR-mix are registered in five different fluorescence channels:

Channel for fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
DNA target	DNA of HPV HR genotype 16	DNA of HPV HR genotype 18	genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	DNA fragment of β-globin gene (IC Glob)	DNA of HPV HR genotype 45
Target gene	E6 gene	E6 gene	E1 gene (for genotypes 16, 31, 33, 35, 52, 58)/ E2 gene (for genotypes 18, 39, 45, 56, 59, 66, 68)/ E7 gene (for genotype 51)	β-globin gene	E6 gene

3 CONTENT

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit (ES3080A) contains:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL HPV 14	clear liquid from colorless to blue grey colour	1.2	1 tube
PCR-buffer-H	colorless clear liquid	0.6	1 tube
DNA calibrator C1 HPV screen	colorless clear liquid	0.2	1 tube
DNA calibrator C2 HPV screen	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	2 tubes

* must be used in the extraction procedure as Negative Control of Extraction

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit is intended for 110 reactions, including controls.

4 ADDITIONAL REQUIREMENTS

- Transport medium.
- Cervical sampler.
- Endocervical brush.
- Gynaecological combined probe.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips (up to 200 µl) and pipette tips with filters (up to 100 µl, 200 µl, 1000 µl).
- Tube racks.
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.

- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments with 5 (or more) independent detection channels (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes:
    - tightly closed 5.0-ml tubes for sampling;
    - tightly closed 1.5 and 5-ml tubes for pretreatment;
    - screwed or tightly closed 1.5-ml tubes for reaction mixture preparation;
    - thin-walled 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;
    - thin-walled 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 with the range from 2 to 8 °C.
- Deep-freezer with the 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 filters and use a new tip for every procedure.
- Store all extracted positive material (samples, 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 afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 a biological cabinet in accordance 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.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in the 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

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (vaginal swab, epithelial scrape from the cervical mucous membrane).

Sampling

Vaginal swab. The material should be obtained from the posterolateral vaginal vault by the cotton swab or combined probe into a tube with transport medium (**Transport Medium with Mucolytic Agent** (952-CE) or PreservCyt (Hologic Inc., USA)). Turn the swab while rubbing it against the surface of the lateral vaginal wall. Collect as much of the material as possible by the swab. The minimal presence of impurities such as mucus and blood is allowed. Transfer the swab into a tube with the transport medium. Break off the lower part of the swab and leave it in the tube with transport medium. In case of impossibility of breaking off the lower part of the swab, the biological material should be washed into the tube with the transport media as much as possible. To do this, press the swab to the interior wall of the tube and rotate it 5-10 times in clockwise and contraclockwise order. The use of pair of scissors is unallowable for cutting-off the lower part of the swab!

Tightly cap the tube avoiding an airspace formation and deformation of the interior part of the cap. Mark the tube. If the **Transport Medium with Mucolytic Agentt** (952-CE) is used its color can be changed due to the change of pH.

Store and transport the biological material transferred in the transport medium according to the requirements specified in the instruction manual for the used transport medium. Only one freeze-thawing cycle is allowed.

Epithelial scrape from the cervical mucous membrane. Allow access to the cervix using disposable or nondisposable sterile gynecological speculum. Carry out the sampling using the cervical brush into the tube with transport medium. Before the specimen collection remove the mucous and vaginal discharge from cervix surface by a gauze tampon. The minimal presence of impurities such as mucus and blood is allowed.

Methods of epithelial scraping:

Method 1. The combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix is used. Place the cervical epithelial scrape (endocervix and ectocervix) to the 5-ml tube with previously added **Transport Medium with Mucolytic Agent** (952-CE).

Method 2. The DNAPAP Cervical Sampler (QIAGEN, Germany) consists of a cervical cytobrush and a tube with Digene transport medium. Place the cervical epithelial scrape (endocervix) to the tube with transport medium.

Method 3. The sampling kit consists of a combined gynecological probe for simultaneously taking epithelial samples from endocervix and ectocervix and a liquid-based cytology vial with PreservCyt transport medium (Hologic Inc., USA). Place the cervical epithelial scrape (endocervix and ectocervix) to the tube with transport medium.

Break off the lower part of the swab and leave it in the tube with transport medium. In case of impossibility of breaking off the lower part of the swab, the biological material should be washed into the tube with the transport media as much as possible. To do this, press the swab to the interior wall of

the tube and rotate it 5-10 times in clockwise and contraclockwise order. The use of pair of scissors is unallowable for cutting-off the lower part of the swab!

Tightly cap the tube avoiding an airspace formation and deformation of the interior part of the cap. Mark the tube.

Store and transport the biological material transferred in the transport medium according to the requirements specified in the instruction manual for the used transport medium. Only one freeze-thawing cycle is allowed.

The biological samples can be stored before the PCR-analysis:

at the temperature from 18 to 25 °C – no more than 7 days;

at the temperature from 2 to 8 °C – no more than 3 months;

at the temperature from minus 24 to minus 16 °C – for 1 year. Only one freeze-thawing cycle is allowed;

in the transport medium for liquid-based cytology at the temperature from 18 to 25 °C – for 1 year.

Pretreatment

The pretreatment is not required for the vaginal swabs and cervical scrapes, collected into the **Transport Medium with Mucolytic Agent** or Digene transport medium.

The pretreatment is required for the cervical scrapes, collected into the transport medium for liquid-based cytology (epithelial cells concentrating).

NOTE: Take an aliquot of cells for the PCR-analysis using only disposable filter tips and disposable tube. It is important to take first an aliquot of cells for the PCR-analysis and then for the liquid-based cytology.

Epithelial cells concentrating

Method 1

1. Take the required number of disposable 5-ml tubes equal to the number of test sample. Mark the tubes. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration. Then gently open and transfer 2-5 ml of cells (depending on density of cells suspension) into the prepared tubes.
2. Leave the tubes in the rack at the temperature from 18 to 25 °C for the night for cells sedimentation and centrifuge on microcentrifuge for **10 min** at **600 g** (for example, **3,000 rpm** for microcentrifuge MiniSpin, Eppendorf).
3. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 1000-µl filter tip for each sample and pipette.
4. Transfer gently the rest of the cell pellet with supernatant (~1 ml) into a new one 1.5-ml tube using a new one filter tip for each sample. Mark the tubes and centrifuge at **10,000 g** (for example, **12,000 rpm** for microcentrifuge MiniSpin, Eppendorf) for **2 min**.
5. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 200-µl filter tip for each sample and vacuum aspirator. Leave **100-200 µl of pellet** with supernatant.

Method 2

1. Shake intensively each vial with the sample for liquid-based cytology for cells disintegration and leave for the night for cells sedimentation.
2. Transfer 0.5-1.0 µl of cells from the bottom of the vial into a new one 1.5-ml tube using 1000-µl tip and pipette. Mark the tube.

3. Centrifuge at **10,000 g** (for example, **12,000 rpm** for microcentrifuge MiniSpin, Eppendorf) for **2 min**.
4. Remove the supernatant from each tube. Do not disturb the cell pellet. Use a new one 200-µl filter tip for each sample and vacuum aspirator. Leave **100-200 µl of pellet** with supernatant.

Interfering substances and limitations of using test material samples

The excessive amount of impurities in biological material such as mucus, blood and pus can lead to the amplification reaction inhibition.

7 WORKING CONDITIONS

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA Extraction

Any commercial DNA extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

DNA-sorb-AM (K1-12-100-CE) for DNA extraction from vaginal swabs and cervical scrapes

- For the automatic extraction

ePure STD DNA Extraction Kit (E2007)

Please carry out nucleic acid extraction according to the manufacture´s instruction.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the type of PCR real-time instrument.

Use disposable tips for adding reagents, DNA and control samples into tubes.

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

1. Calculate the volume of each reagent required for preparation of 4 reaction mixtures. **10 µl** of **PCR-mix-FL HPV 14** and **5 µl** of **PCR-buffer-H** are required for 1 reaction. Prepare the mixture for the total number of test and control samples (see item 7 for the number of control samples) plus extra volume for several reactions.
   NOTE: Reaction mixture components should be mixed just before analysis.
2. Thaw the tube with **PCR-mix-FL HPV 14**. Vortex the tubes with **PCR-mix-FL HPV 14, PCR-buffer-H** and then centrifuge briefly.
3. To prepare the reaction mixture, mix the required quantity of **PCR-mix-FL HPV 14** and **PCR-buffer-H** in a new sterile tube. Sediment the drops on vortex.
4. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
5. Transfer **15 µl** of the prepared mixture to each tube. Utilize the rest of reaction mixture.
6. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage from test samples to the prepared tubes.

NOTE: Avoid transferring sorbent beads together with the DNA sample in case of extraction using reagent kit with sorption on silica gel or magnetic separation.

7. Carry out the control amplification reactions:

C1	Add 10 µl of DNA calibrator C1 HPV screen to the tube with reaction mixture
C2	Add 10 µl of DNA calibrator C2 HPV screen to the tube with reaction mixture
C-	Add 10 µl of the sample extracted from the Negative Control (C-) reagent to the tube with reaction mixture

NOTE: It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination.

NCA – Add **10 µl** of **TE-buffer** to the tube with reaction mixture

8.2.2 Amplification

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

Table 1

eSens amplification program

Unified amplification program for rotor instruments (e.g. Rotor-Gene Q/QIAGEN, Germany) and plate instruments (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.).

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	20 s	FAM, JOE, ROX, Cy5, Cy5.5	

If only the tests for pathogen agent DNA detection are performed in one instrument, then the first step of reverse transcription (50 °C – 15 minutes) can be omitted for time saving.

Table 2

eSens-1 amplification program

	Rotor-type instrument (e.g. Rotor-Gene Q / QIAGEN, Germany, or equivalent)			Plate-type instrument (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40

	60	20 s Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

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

NOTE: eSens-1 is an universal program for conducting tests for identifying human papillomaviruses (HPV HR) and detection of STIs and other infections of reproductive system with eSens PCR kits. Therefore, any combination of these tests can be carried out simultaneously in the same instrument.

2. Adjust the fluorescence channel sensitivity according to the Technical Sheet.
3. Insert tubes into the reaction module of the device.
   NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument. Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

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:

Table 3

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 HR (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 DNA sample in the corresponding column of the results grid.

The calibration curve is automatically plotted on the basis of obtained threshold *Ct* values and known calibrators (C1 and C2) values, and human DNA and HPV DNA concentrations (copies) are calculated. Obtained values are used for calculation of HPV DNA quantity per $1x10^5$ human cells. Normalized values reflect the number of copies of the pathogen relative to human cells. Moreover the concentration values of human DNA allows to estimate the quality of biological material sampling.

Obtained data are used for calculation of HPV DNA quantity according to the formula:

$$\lg\left(\frac{\text{number of HPV DNA copies in 1 ml}}{\text{number of human DNA copies in 1 ml}} \times 2*10^5\right) = \lg(\text{HPV DNA copies} / 10^5 \text{ human cells})$$

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.

NOTE:

- Concentration values of calibrators are specified in the Technical Sheet enclosed to the PCR kit.
- For the subsequent runs with the given lot of the eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit one can use the results of DNA calibrator C1 obtained in the previous run on this instrument. For that purpose export the results of DNA calibrator C1 using the software of the instrument. In this case the run only for the DNA calibrator C1 is required.

For convenience in operation it is recommended to use the template for results calculation in Microsoft Excel format **"eSens HPV HR 14 screen/16,18,45 genotype QT.xls"**

Observe the following conditions for automatic data processing and obtaining the valid results:

1. Complete the table correctly with the respective designation of the control samples.
2. Make sure that Microsoft Excel security system allows to run macros. Set the *Middle Security Level* in the *Service>Macro>Security* menu.

Automatic processing of data

1. Open the file with the calculation template and agree to enable macros.
2. Make sure that the *Calculate* button is activated.
3. If the table is completed with data from the previous run it can be cleaned by the *Clear the table* button.
4. Copy the *Ct* values from the instrument software or the file of data export into the excel table.
5. Mark the control samples in accordance with Acceptable Sample Names.
6. Use any letter case.
7. Mark the empty cells (analysis is not performed) by # symbol. To do this, click the *Mark Unnamed Samples as Empty* button.
8. Complete the information about the run.
9. Save the file with the different name.
10. Click the *Calculate* button. Data of calibration lines, calculated concentrations, results (quantitative result, interpretation) will be displayed in the file.
11. Make sure that the calibration was successfully performed: the *OK* message is displayed for each channel in the *Calibration status* column of the *Calibration* table.
12. View the samples status, pay special attention to the control samples.
13. Perform results interpretation according to the Table 4.

Using the results obtained for DNA calibrator C1 in the previous run

Complete the excel table, click the *Calculate* button. Check the calibration status (the *OK* message should be displayed for each channel in the *Calibration status* column of the *Calibration* table) and click the *Save Calibration* button.

In the next run complete *the excel table* with data obtained for test samples, DNA calibrator C2, negative controls, and click the *Use the calibration* button. The saved *Ct* values will appear in the underneath cells marked in grey. Then click the *Calculate* button as described above.

Table 4

Results interpretation for the test samples

Result	Interpretation
Invalid (insufficient amount of biological material)	DNA concentration of IC Glob (obtained for samples in the channel for Cy5 fluorophore) is less than $1x10^5$ copies/ml (500 cells/reaction) and the calculated concentration values of HPV HCR DNA 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
HPV HR DNA is not detected	The *Ct* value for HPV HCR DNA is absent and the concentration of IC Glob is more than $1x10^5$ copies/ml (500 cells/reaction). The result is HPV HR DNA is not detected
<3 lg (HPV per 10^5 human cells)	Clinically insignificant value
3–5 lg (HPV per 10^5 human cells)	Clinically significant value. Dysplasia cannot be excluded; risk of dysplasia development
>5 lg (HPV per 10^5 human cells)	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 results of the analysis is considered reliable only if the results obtained for controls of amplification and extraction stages are correct (according to Table 5 and the Technical Sheet enclosed to the PCR kit).

Table 5

Results for controls

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

10 TROUBLESHOOTING

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

1. The *Ct* value is determined for the Negative Control of Extraction (C-) in the channels for the FAM and/or JOE and/or ROX and/or Cy5 and/or Cy5.5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable

at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.

2. The *Ct* value is absent for the DNA-calibrator C1, C2 in any detection channel (see Table 5). It is necessary to repeat the amplification and detection for all the samples.
3. The efficiency E is less than 80 % or greater than 120 % when plotting the calibration curve. Check the correctness of set concentrations of DNA-calibrators in accordance with the *Technical Sheet* and the correctness of selected level of the threshold line. If set concentrations of DNA-calibrators and the threshold line level are correct but the efficiency does not fit in the required range, then the amplification and detection for all the samples should be repeated.
4. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12 STABILITY AND STORAGE

All components of the **eSens HPV HR 14 screen/16,18,45 genotype** QT PCR kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **eSens HPV HR 14 screen/16,18,45 genotype QT 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.

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

13 SPECIFICATIONS

13.1 Analytical sensitivity and linear range

Biological material	Transport medium	Nucleic acid extraction kit	PCR kit	Microorganism	Analytical sensitivity, copies/ml*	Linear measure-ment range, copies/ml
Vaginal swab, epithelial scrape from the cervical mucous membrane (ectocervix and endocervix)	Transport Medium with Mucolytic Agent, TC Digene or Transport medium for liquid-based cytology	DNA-sorb-AM, ePure STD DNA Extraction Kit	ES3080A	HPV HCR genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	$5x10^3$	$7x10^3$ – $1x10^8$

* Number of copies of virus DNA in the biological material placed in the specified transport medium and calculated per 1 ml.

The claimed features are achieved while respecting the rules specified in the section *Sampling and Handling*.

13.2 Analytical specificity

The analytical specificity of **eSens HPV HR 14 screen/16,18,45 genotype QT 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 HPV HR genotypes. 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* spp., *Mycoplasma hominis, Ureaplasma parvum, Streptococcus agalactiae, Streptococcus pyogenes, Candida* spp., *Cytomegalovirus, EBV, Varicella-Zoster virus, HSV* I, *HSV* II, *Human papillomavirus* of low and unknown risk (genotypes 6, 11, 67, 70, 84, 81, 82, 62, 72, 73) were used. Nonspecific responses as well as cross-reactions between HPV genotypes when using highly concentrated samples were absent.

The clinical specificity of **eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit** was confirmed in laboratory clinical trials.

13.3 Reproducibility, repeatability and trueness

Repeatability and reproducibility were determined by testing of quality control samples with 3 concentration ranges (from $7x10^3$ to $1x10^4$, from $1x10^4$ to $1x10^6$, from $1x10^6$ to $1x10^8$ 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)	Coefficient of variation (CV), %
16	from $7x10^3$ to $1x10^4$	16	3.55	0.04	1.19
18		16	3.94	0.07	1.71
31		16	3.73	0.09	2.55
33		16	3.68	0.13	3.44
35		16	3.45	0.15	4.30
39		16	3.66	0.12	3.32
45		16	3.65	0.06	1.58
51		16	3.50	0.14	4.09
52		16	3.64	0.11	2.96
56		16	3.78	0.13	3.36
58		16	3.79	0.11	2.86
59		16	3.46	0.09	2.46
66		16	3.88	0.09	2.35
68		16	3.60	0.13	3.53
16	from $1x10^4$ to $1x10^6$	16	5.93	0.11	1.93
18		16	6.04	0.07	1.23
31		16	3.94	0.18	4.61
33		16	4.35	0.13	2.88
35		16	5.61	0.12	2.16
39		16	5.18	0.12	2.22
45		16	5.77	0.15	2.66
51		16	5.60	0.17	3.03
52		16	5.37	0.20	3.68
56		16	5.80	0.23	3.90
58		16	5.64	0.09	1.56
59		16	5.19	0.12	2.33

HPV genotype	Initial concentration value, lg copies/ml	Number of repeats	Average concentration value, lg copies/ml	Standard deviation (SD)	Coefficient of variation (CV), %
66		16	5.41	0.14	2.54
68		16	5.18	0.03	0.59
16	from $1x10^6$ to $1x10^8$	16	7.71	0.50	6.41
18		16	7.92	0.09	1.19
31		16	6.30	0.23	3.72
33		16	6.40	0.05	0.72
35		16	7.60	0.27	3.51
39		16	7.23	0.10	1.35
45		16	7.80	0.22	2.81
51		16	7.48	0.08	1.04
52		16	7.50	0.14	1.86
56		16	7.64	0.16	2.10
58		16	7.52	0.04	0.54
59		16	6.70	0.37	5.46
66		16	7.30	0.28	3.83
68		16	7.10	0.04	0.55

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	from $7x10^3$ to $1x10^4$	8	3.56	0.04	1.18
18		8	3.91	0.06	1.50
31		8	3.78	0.05	1.19
33		8	3.57	0.03	0.95
35		8	3.33	0.07	2.18
39		8	3.56	0.08	2.19

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), %
45		8	3.61	0.03	0.84
51		8	3.37	0.04	1.09
52		8	3.58	0.10	2.76
56		8	3.68	0.08	2.13
58		8	3.70	0.08	2.07
59		8	3.41	0.08	2.40
66		8	3.81	0.06	1.61
68		8	3.51	0.07	2.12
16	from $1x10^4$ to $1x10^6$	8	5.88	0.13	2.19
18		8	5.99	0.07	1.13
31		8	3.89	0.24	6.21
33		8	4.46	0.02	0.49
35		8	5.71	0.08	1.38
39		8	5.08	0.09	1.77
45		8	5.67	0.15	2.60
51		8	5.75	0.06	0.97
52		8	5.73	0.08	1.43
56		8	5.64	0.23	4.06
58		8	5.58	0.08	1.44
59		8	5.13	0.08	1.64
66		8	5.28	0.04	0.75
68		8	5.18	0.03	0.58
16	from $1x10^6$ to $1x10^8$	8	8.04	0.11	1.38
18		8	8.01	0.04	0.54
31		8	6.50	0.05	0.77
33		8	6.40	0.03	0.54
35		8	7.38	0.05	0.67
39		8	7.33	0.03	0.41

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), %
45		8	8.00	0.05	0.68
51		8	7.45	0.07	0.98
52		8	7.26	0.05	0.71
56		8	7.67	0.18	2.34
58		8	7.55	0.02	0.24
59		8	6.37	0.05	0.80
66		8	7.05	0.15	2.14
68		8	7.14	0.03	0.38

The trueness was determined by testing the quality control samples with the concentration of at least $7x10^3$ copies/ml.

Table 8

Trueness

HPV genotype	Number of repeats	Average value of measurement, lg	Specified value, lg	Bias (B), %
16	40	4.13	4.25	2.86
18	40	4.28	4.45	3.96
31	40	4.42	4.28	3.35
33	40	4.60	4.44	3.56
35	40	4.29	4.46	3.80
39	40	4.07	4.33	6.20
45	40	4.10	4.44	7.69
51	40	4.34	4.40	1.38
52	40	4.41	4.45	0.81
56	40	4.30	4.32	0.55
58	40	4.30	4.38	1.77
59	40	4.10	4.47	8.12
66	40	4.62	4.52	2.21
68	40	4.40	4.41	0.30

13.4 Diagnostic characteristics

Diagnostic characteristics of **eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit** were determined according to international requirements for validation of new tests for HPV DNA detection (Meijer CJ, et al. 2009. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in woman 30 years and older/ Int. J. Cancer 124:516-520.):

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 900 samples (epithelial scrape from the cervical mucous membrane) were studied to determine diagnostic sensitivity and specificity of the kit. The 100 of these samples are with histologically confirmed diagnosis of CIN2+ and the average age of female patients is 35 years old (from 30 to 65 years old). And 800 of all samples obtained from screening study are with cytologically/histologically confirmed absence of CIN2. An average age of the women is 39 years old (from 30 to 65 years old).

Moreover 300 samples of vaginal mucosal swabs obtained from screening study were studied.

Table 9

The results of testing eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit in comparison with the reference assay

Samples type	The results of application of eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit		Results of using the reference assay*	
			Positive	Negative
Scrape from the cervical mucous membrane (ectocervix and endocervix) with histologically confirmed moderate or severe dysplasia (CIN2+)	100 samples were tested	Positive	100	0
		Negative	0	0
Scrape from the cervical mucous membrane (ectocervix and endocervix) with normal cytology or mild dysplasia	800 samples were tested	Positive	156	0
		Negative	0	644
Vaginal swab	300 samples were tested	Positive	147	0
		Negative	0	153

* As reference methods

- Digene hc2 High-Risk HPV DNA Test kit (for epithelial scrape from the cervical mucous membrane (ectocervix and endocervix)),
- AmpliSens® *HPV* HCR screen-titre-FRT PCR kit (REF R-V31-T-4x(RG,iQ,Mx)-CE), AmpliSens® *HPV* HCR genotype-FRT PCR kit (REF R-V25(RG,iQ,Mx)-CE) (for all types of samples) and
- Sanger sequencing of L1 region of HPV genome (for discordant samples) were used.

Table 10

Diagnostic characteristics of eSens HPV HR 14 screen/16,18,45 genotype QT PCR kit

Samples type	Diagnostic sensitivity*[1], in the interval (%)	Diagnostic specificity*[2], in the interval (%)
Scrape from the cervical mucous membrane (ectocervix and endocervix)	100	100
Vaginal swab	100	100

*[1] Relative sensitivity in comparison with applied reference methods.

*[2] Relative specificity in comparison with applied reference methods.

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.
2. Guidelines to **eSens HPV *HR 14 screen/16,18,45 genotype QT*** PCR kit for detection of the DNA of *human papillomaviruses* (HPV) of high carcinogenic risk (HCR) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 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

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

16 KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	In vitro diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	C1, C2	DNA-calibrators

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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