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For Professional Use Only

eSens Florocenosis/Aerobes QT PCR kit

REF ES3047A

Instructions for Use

1 INTENDED USE

eSens Florocenosis/Aerobes QT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of DNA of *Enterobacteriaceae, Staphylococcus* spp. and *Streptococcus* spp. in the biological material (vaginal swab), in the diagnosis of inflammatory infections of female genitourinary system using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA-samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used in clinical laboratory diagnostics for investigation of biological material taken from the women with suspected of inflammatory infections of genitourinary system.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2 PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. 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 quantitative detection of *Enterobacteriaceae, Staphylococcus* spp. and *Streptococcus* spp. is based on the linear dependence between the initial DNA-target concentration in the sample and the cycle of start of the exponential growth of the fluorescent signal (cycle threshold, *Ct*). For the quantitative analysis amplification of DNA from the test samples is carried out simultaneously with DNA-calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the test samples.

eSens Florocenosis/Aerobes 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.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (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.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
DNA-target	*Enterobacteriaceae*	*Staphylococcus* spp.	*Streptococcus* spp.	IC
Target gene	*gene* 16S rRNA	*gene* 16S rRNA	*gene* 16S rRNA	Artificially synthesized sequence

3 CONTENT

eSens Florocenosis/Aerobes QT PCR kit (ES3047A) includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-FL Florocenosis / Aerobes		clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-buffer-B		colorless clear liquid	0.6	1 tube
Polymerase (TaqF)		colorless clear liquid	0.06	1 tube
TE-buffer		colorless clear liquid	0.2	1 tube
DNA calibrators	**K1 AB**	colorless clear liquid	0.2	1 tube
	K2 AB	colorless clear liquid	0.2	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**		colorless clear liquid	1.0	1 tube

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

****** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

eSens Florocenosis/Aerobes QT PCR kit is intended for 110 reactions (including controls and calibrators).

eSens Florocenosis/Aerobes QT excel (version 1.1.) for automatic processing of data.

4 ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium.
- Universal gynecological swab.
- Flocked swab for sampling, transportation and storage of biological samples.

For DNA extraction and amplification

- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (5 or more 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:

  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.

b) 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;
c) 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 for 2–8 °C.
- Deep-freezer at temperature 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 a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- 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 DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

eSens Florocenosis/Aerobes QT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (vaginal swabs).

Sampling

6.1 Vaginal swabs.

The material should be obtained from the posterolateral vaginal vault by the universal or flocked swab into a tube with transport medium. 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 Agent** (952-CE) is used its color can be changed due to the change of pH (then the discharge is acidic).

Samples can be stored in **Transport Medium with Mucolytic Agent** before the PCR-analysis:

- at the temperature from 18 to 25 °C – for 28 days;
- at the temperature from 2 to 8 °C – for 3 months;
- at the temperature not more than minus 20 °C – for a long time.

Only one freeze-thawing cycle is allowed.

Pretreatment

The pretreatment of the vaginal swabs is not required.

Interfering substances and limitations of using test material samples

The effect of potentially interfering substances was studied on model samples. Model samples were prepared by breeding in **Negative Control (C-)** reagent (included in the **DNA-sorb-AM** nucleic acid extraction kit) of a quality control sample containing Enterobacteriaceae, Staphylococcus spp., Streptococcus spp. DNA to a concentration of $2x10^3$ and $2x10^4$ GE/ml with and without addition of potentially interfering substances.

To assess the effect of high concentrations of exogenous substances The following drugs in different concentrations (see Table 2) were used: «Miramistin®» («Infamed», Russia); «Chlorhexidine bigluconate», 0,05% topical solution («Biogen NPC», Russia); «Clotrimazole», cream 1% («Ozon», Russia); «Metrogyl», vaginal gel (metronidazole 1%), (Unique Pharmaceutical Laboratories, India); «Polygynax», vaginal capsules (Innotech International, France); «Macmiror® complex», vaginal capsules (Polichem Srl, Italy); «Hasico For Women», gel lubricant («EMANSI», Russia); «Contex Silk», silicone gel lubricant for intimate use (Contex, the Czech Republic), «Play Feel», gel lubricant for sensitivity increasing (Durex, Thailand). The concentration of exogenous substances was determined as the volume of the drug to the volume of the test sample. The maximum concentration of exogenous substance corresponded to the maximum possible volume of a vaginal swab placed in the transport medium while sampling of clinical material with probes recommended for use.

Glycogen, hemoglobin, lactoferrin, mucin in various concentrations were used to assess the effect of endogenous substances (see Table 2). The concentrations of endogenous substances were determined

taking into account the literature data on the study of PCR inhibitors. The highest concentration of endogenous substances exceeded more than twice the concentration of PCR inhibiting substances according to literature data.

Table 2

Type of potential interferent	Potential interferent	Tested concentration	Interference presence
Endogenous substances	Haemoglobin	65; 130; 260 µg/ml	Not detected
	Lactoferrin	1.25; 2.5; 5 µg/ml	Not detected
	Glycogen	30; 60; 120 mg/ml	Not detected
	Mucin	50; 100; 150 µg/ml	Not detected
Exogenous substances	«Miramistin®»	4; 8; 16 % (the drug volume to the volume of the test sample)	Not detected
	«Chlorhexidine bigluconate»		Not detected
	«Clotrimazole»		Not detected
	«Metrogyl»		Not detected
	«Polygynax»		Not detected
	«Macmiror® complex»		Not detected
	«Hasico For Women»		Not detected
	«Contex Silk»		Not detected
	«Play Feel»		Not detected

The absence of the effect of the studied potentially interfering substances is guaranteed only when using the **DNA sorb-AM** nucleic acid extraction kit.

In order to control the efficiency of DNA extraction and amplification the Internal Control (Internal Control STI-87 (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

7 WORKING CONDITIONS

eSens Florocenosis/Aerobes QT PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid 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 the automatic extraction

- **ePure Bacterial DNA Extraction Kit** (E2006)

NOTE: Extract the DNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC)**.
Extraction of DNA by express methods is unallowable.

8.2 Preparing RCR

8.2.1 Preparing tubes for RT-RCR

The type of tubes depends on the type of PCR real-time instrument. Use disposable filter 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**.

NOTE: Components of the reaction mixture should be mixed just before the PCR studies.

1. Vortex the tubes with **PCR-mix-FL Florocenosis / Aerobes, PCR-buffer-B** and **polymerase (TaqF)** and sediment the drops by short centrifugation.
2. Take the required number of tubes or strips for amplification of the DNA obtained from test and control samples.
3. For 1 reaction, add to a new tube (see table 3):
   10 µl of **PCR-mix-FL Florocenosis / Aerobes,**
   5.0 µl of **PCR-buffer-B** and **polymerase (TaqF)**.

Previously, prepare the mixture of **PCR-buffer-B** and **polymerase (TaqF)**. To do this, transfer the entire content of the tube with **polymerase (TaqF) (60 µl)** into the tube with **PCR-buffer-B (600 µl).** Vortex the tube carefully avoiding foaming. Mark the tube with the mixture preparation date.

NOTE: The prepared mixture is intended for 110 samples. The mixture can be stored at 2–8 °C for 3 months and used when it is necessary.

Table 3

Scheme of reaction mixture preparation

Reagent volume for specified number of reactions, µl (Values are specified including one extra reaction and four control reactions: DNA calibrators K1, K2, C– and NCA.)		
Reagent volume per one reaction, µl	**10.0**	**5.0**
Number of biological samples to be tested	**PCR-mix-FL Florocenosis / Aerobes**	**Mix of PCR-buffer-B and polymerase (TaqF)**
1	**60**	**30**
2	**70**	**35**
3	**80**	**40**
4	**90**	**45**

Reagent volume for specified number of reactions, µl (Values are specified including one extra reaction and four control reactions: DNA calibrators K1, K2, C– and NCA.)		
Reagent volume per one reaction, µl	**10.0**	**5.0**
Number of biological samples to be tested	**PCR-mix-FL Florocenosis / Aerobes**	**Mix of PCR-buffer-B and polymerase (TaqF)**
5	**100**	**50**
6	**110**	**55**
7	**120**	**60**
8	**130**	**65**
9	**140**	**70**
10	**150**	**75**
11	**160**	**80**
12	**170**	**85**
13	**180**	**90**
14	**190**	**95**
15	**200**	**100**
16	**210**	**105**
17	**220**	**110**
18	**230**	**115**
19	**240**	**120**
20	**250**	**125**
21	**260**	**130**
22	**270**	**135**
23	**280**	**140**
24	**290**	**145**
25	**300**	**150**
30	**350**	**175**

4. Transfer **15 µl** of the prepared mixture into each tube. Dispose the remaining mixture.
5. Using tips with aerosol filter, add **10 µl** of DNA samples obtained at the DNA extraction stage.

NOTE: Avoid transferring of sorbent together with the DNA samples extracted by **DNA-sorb-AM** kit.

6. Carry out the control reactions:

NCA	–	Add **10 µl** of **TE-buffer** into the tube labeled NCA (Negative Control of Amplification).
DNA calibrator K1	–	Add **10 µl** of **DNA calibrator K1 AB** into the tube labeled K1.
DNA calibrator K2	–	Add **10 µl** of **DNA calibrator K2 AB** into the tube labeled K2.
C–	–	Add **10 µl** of the sample extracted from **Negative Control (C–)** reagent into the tube labeled C– (Negative Control of Extraction).

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows (see Table 4, 5). The amplification programs (tables 4 and 5) are equal for the given PCR kit.:

Table 4

eSens unified amplification program

	Rotor-type instruments (For example, Rotor-Gene Q (QIAGEN, Germany) or equivalent.)		**Plate-type instruments** (For example, CFX 96 (Bio-Rad, USA) or equivalent.)	
Step	**Temperature, °C**	**Time**	**Fluorescent signal detection**	**Cycle repeats**
1	**50**	**15 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**20 s**	**FAM, JOE, ROX, Cy5**	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in “multiprime” format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If only the tests for DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving.

Table 5

eSens-1 amplification program

	Rotor-type instruments (For example, Rotor-Gene Q (QIAGEN, Germany) or equivalent.)			**Plate-type instruments** (For example, CFX 96 (Bio-Rad, USA) 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** and **Cy5** fluorophores.

NOTE: Channel for the Cy5.5 fluorophore is enabled when necessary, if tests in "multiprime" format (that use this channel) are carried out.

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

NOTE: In case of incomplete loading of plate-type instrument it is necessary to insert the empty tubes at the borders of the thermocycler reaction module.

3. Run the amplification program with fluorescence detection.
4. 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 four channels:

Table 6

Channel for the fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
Signal registration, indicating the amplification product accumulation	*Enterobacteriaceae* DNA	*Staphylococcus* spp. DNA	*Streptococcus* spp. DNA	Internal Control-FL (IC) DNA

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.

Based on the obtained *Ct* values and specified concentration values of K1 AB and K2 AB DNA calibrators a calibration line is plotted automatically and it is used for the calculation of number of copies of *Enterobacteriaceae*, *Staphylococcus* spp. and *Streptococcus* spp. DNA concentration for test and control samples.

NOTE: The values of DNA calibrators' concentrations are specified in the *Technical Sheet* enclosed to the PCR kit.

The obtained values are used for calculation of number of genome equivalents (GE) of corresponding microorganisms DNA per 1 ml of the biological sample according to the formula:

Number of microorganisms DNA copies x K =
Number of genomic equivalents per 1 ml (GE/ml)

NOTE: The coefficient **K** for calculation of the result in GE/ml is specified in the *Technical Sheet* enclosed to the PCR kit.

The concentration values of *Enterobacteriaceae*, *Staphylococcus* spp. and *Streptococcus* spp. DNA indicate the total content of the microorganisms in the biological material transferred into transport medium.

If the obtained value is less than $1x10^4$ GE/ml then the result is interpreted as "less than $1x10^4$ GE/ml", if the obtained value is greater than $1x10^8$ GE/ml then the result is interpreted as "greater than $1x10^8$ GE/ml" (according to the linear range of the kit).

The result of analysis is considered **invalid** if the *Ct* value for the sample in the channel for the Cy5 fluorophore is absent or determined greater than the boundary value, herewith the calculated values of concentrations of *Enterobacteriaceae*, *Staphylococcus* spp. or *Streptococcus* spp. DNA are not defined or less than 10^4 GE/ml. PCR should be repeated for this sample starting from the DNA extraction stage.

***Enterobacteriaceae*, *Staphylococcus* spp. and *Streptococcus* spp. DNA is not detected** if the *Ct* value for the sample for *Enterobacteriaceae*, *Staphylococcus* spp. or *Streptococcus* spp. DNA is absent, and the *Ct* value determined in the channel for the Cy5 fluorophore is less than the boundary value.

NOTE: Boundary *Ct* values are specified in the *Technical Sheet* enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results obtained for Negative and Positive Controls of extraction and amplification are correct (in accordance with table 7 and *Technical Sheet* enclosed to the PCR kit).

Table 7

Results for controls

Control	Stage for control	Amplification results in the channel for the fluorophore			
		FAM	**JOE**	**ROX**	**Cy5**
C–	DNA Extraction	Concentration value is absent or < boundary value	Concentration value is absent or < boundary value	Concentration value is absent or < boundary value	*Ct* value < boundary value
NCA	PCR	Concentration value is absent or < boundary value	Concentration value is absent or < boundary value	Concentration value is absent or < boundary value	*Ct* value is absent
K1	PCR	*Ct* value is determined	*Ct* value is determined	*Ct* value is determined	*Ct* value is determined
K2	PCR	*Ct* value < boundary value	*Ct* value < boundary value	*Ct* value < boundary value	*Ct* value < boundary value

10 TROUBLESHOOTING

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

1. The cycle threshold (*Ct*) values for the DNA calibrators (K1, K2) in the channels for the FAM and/or JOE and/or ROX fluorophores are absent, or the *Ct* values of DNA calibrator K2 exceed the boundary value, or the efficiency coefficient E according to the standard plot is less than the value specified in the *Technical Sheet*. The amplification and detection should be repeated for all the samples.
2. The calculated value of concentration (GE/ml) of *Enterobacteriaceae, Staphylococcus* spp. and *Streptococcus* spp. DNA for the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) is greater than the boundary value specified in the *Technical Sheet*. PCR should be repeated for all the samples from the DNA extraction stage.

11 TRANSPORTATION

eSens Florocenosis/Aerobes QT PCR kit should be transported at 2–8 °C for no longer than 5 days. Transportation at 2-25°C for no longer than 3 days is allowed.

12 STABILITY AND STORAGE

All components of the **eSens Florocenosis/Aerobes QT PCR kit** are to be stored at 2–8 °C when not in use (except for polymerase (TaqF) and PCR-buffer-B). All components of the **eSens Florocenosis/Aerobes QT PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-buffer-B are to be stored at temperature from minus 24 to minus 16 °C.

PCR-mix-FL Florocenosis / Aerobes is to be kept away from light.

13 SPECIFICATIONS

13.1 Linear measurement range and analytical sensitivity (detection limit)

Table 8

Test material	Transport medium	Nucleic acid extraction kit	Microorganisms	Analytical sensitivity (detection limit), GE/ml*	Linear measure-ment range, GE/ml
Vaginal swab	Transport Medium with Mucolytic Agent	DNA-sorb-AM	*Enterobacteriaceae*	$2x10^3$	$1x10^4 – 1x10^8$
			Staphylococcus spp.		
			Streptococcus spp.		

* Number of genome equivalents (GE) of the pathogen agent in the biological material (vaginal swab) transferred into the transport medium calculated per 1 ml.

13.2 Analytical specificity

The analytical specificity of **eSens Florocenosis/Aerobes 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 nonspecific reactions were absent while testing:

- human DNA with concentration $5x10^7$ GE/ml;
- DNA of the following microorganisms from ATCC collection (American Type Culture Collection, USA) and clinical isolates with concentration $1x10^7$ GE/ml: *Lactobacillus* spp., *Gardnerella vaginalis* ATCC® 14018™, *Enterococcus faecium* ATCC® 35667™, *Neisseria gonorrhoeae* ATCC® 49926™, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Candida albicans* ATCC® 14053, *HSV* (herpes simplex virus) types 1 and 2, *CMV* (cytomegalovirus).

During testing the samples of DNA of microorganisms belonging to *Enterobacteriaceae, Staphylococcus* spp. and *Streptococcus* spp., including DNA of the following microorganisms from ATCC collection and clinical isolates with concentration no less than $7x10^7$ GE/ml: *Escherichia coli* ATCC® 25922™, *Klebsiella pneumoniae* ATCC® 27736™, *Proteus mirabilis* ATCC® 12453™, *Staphylococcus aureus* ATCC® 6538P™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus saprophyticus* ATCC® 4990™, *Streptococcus agalactiae* ATCC® 12386™, *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus pyogenes* ATCC® 19615™, the positive results were obtained for each group only in the channel for detection of DNA of corresponding microorganisms group and non-specific results were absent in other channels.

The clinical specificity of **eSens Florocenosis/Aerobes QT PCR kit** was confirmed in laboratory clinical trials.

The information about known interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3 Reproducibility, repeatability and trueness

The reproducibility and repeatability were determined by testing the model samples of biological material. The model samples of biological material were prepared by dilution of quality control sample containing *Enterobacteriaceae, Staphylococcus* spp., *Streptococcus* spp. DNA with concentrations $1x10^4$, $1x10^6$ and $1x10^8$ GE/ml in the biological material – vaginal swabs (transferred into **Transport Medium with Mucolytic Agent**). The pool samples of vaginal swabs, in which the content of DNA of microorganisms groups detected by the PCR kit does not exceed $1x10^4$ GE/ml, is used for the model samples preparation. Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing in different laboratories, by different operators, using different equipment.

Table 9

Reproducibility

Microorganism	Initial concentration value, GE/ml	Average number of repeats	Average concentration value, lg	Average standard deviation (SD)	Average coefficient of variation (CV), %
Enterobacteriaceae	$1x10^4$	20	4.35	0.15	3.52
Staphylococcus spp.		20	4.38	0.16	3.62
Streptococcus spp.		20	4.37	0.16	3.63
Enterobacteriaceae	$1x10^6$	20	6.40	0.05	0.81
Staphylococcus spp.		20	6.20	0.02	0.33
Streptococcus spp.		20	6.42	0.04	0.56
Enterobacteriaceae	$1x10^8$	20	8.33	0.18	2.18
Staphylococcus spp.		20	8.37	0.17	2.00
Streptococcus spp.		20	8.35	0.15	1.80

Table 10

Repeatability

Microorganism	Initial concentration value, GE/ml	Average number of repeats	Average concentration value, lg	Average standard deviation (SD)	Average coefficient of variation (CV), %
Enterobacteriaceae	$1x10^4$	40	4.33	0.17	3.80
Staphylococcus spp.		40	4.35	0.16	3.72
Streptococcus spp.		40	4.35	0.16	3.69
Enterobacteriaceae	$1x10^6$	40	6.40	0.07	1.01
Staphylococcus spp.		40	6.20	0.04	0.71
Streptococcus spp.		40	6.42	0.05	0.72
Enterobacteriaceae	$1x10^8$	40	8.32	0.17	2.11
Staphylococcus spp.		40	8.36	0.17	1.99
Streptococcus spp.		40	8.36	0.14	1.68

The trueness was determined by measuring the quantitative DNA content of the three determined groups of bacteria in the dilution of quality control sample (QCS) with a concentration of $5x10^4$ GE/ml.

Table 11

Trueness

Microorganism	Number of repeats	Average value of measurement, lg	Average specified value	Average bias (B), %
Enterobacteriaceae	100	4.77	4.70	1.49
Staphylococcus spp.	100	4.68	4.72	0.83
Streptococcus spp.	100	4.66	4.70	1.57

14 QUALITY CONTROL

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

15 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	**NCA**	Negative control of amplification
	Date of manufacture	**C-**	Negative control of extraction
EC REP	Authorized representative in the European Community	**K1, K2**	DNA-calibrators
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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