DNA-TECHNOLOGY

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For professional use only

Femoflor® Screen REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **Femoflor® Screen REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Femoflor® Screen REAL-TIME PCR Detection Kit** is an in vitro Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Femoflor® Screen REAL-TIME PCR Detection Kit** is intended to detect pathogens, opportunistic flora and normal flora in women's urogenital specimens by multiplex Real-Time PCR method. Samples are human biological materials: epithelial scrapes from cervical canal, posterolateral vaginal vault and urethra.

The **Femoflor® Screen REAL-TIME PCR Detection Kit** offers simultaneous, comprehensive diagnostics, using one biological sample:

- qualitative detection of pathogens of genitourinary diseases in women (*Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, Cytomegalovirus, Herpes simplex virus 1, Herpes simplex virus 2, involved in the development of dysbiotic processes in urogenital microbiocenosis);

- microbiota composition assessment: urogenital normoflora - lactobacilli, obligate-anaerobic microorganisms - *Gardnerella vaginalis*/*Prevotella bivia*/*Porphyromonas* spp., typical for the women's urogenital tract, mycoplasmas - *Mycoplasma hominis*, *Ureaplasma* spp., fungi of the Candida genus.

The microbiome state has a serious impact on the reproductive function and, as a result, on the quality of woman's life. Diseases caused by opportunistic microflora may occur both with clinical manifestations and asymptomatically. The asymptomatic disease course often leads to late reference to doctor and development of serious complications. Diseases caused by opportunistic microorganisms increase the risk of infection with sexually transmitted infections and HIV infections. In time undiagnosed infections, associated with opportunistic microflora, can cause abnormalities in the women reproductive function, spontaneous abortions, premature births, intrauterine infection and low fetal birth weight, postnatal complications, and complications after surgery intervention on the pelvic organs. The urgency of diagnostics of the opportunistic microflora associated infections of urogenital tract is in question. The diagnostic methods used in routine laboratory practice do not always allow the doctor to adequately assess the patient's condition and prescribe the necessary treatment. At the same time, the possibilities of the modern PCR laboratory allow to conduct the multifactorial studies, detecting the DNA of various microorganisms in the samples.

There are no contradictions for use of the **Femoflor® Screen REAL-TIME PCR Detection Kit.**

The **Femoflor® Screen REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. According to cyclic amplification DNA is denatured by heating. Target specific primers bind to the denatured DNA templates in the presence of dNTP's, and Taq-polymerase. Taq-polymerase extends the primers thus providing the synthesis of complementary DNA chains and amplification of target DNA sequence.

To increase the specificity and sensitivity of the amplification reaction, a paraffin layer separates the PCR-mix and Taq-polymerase in standard package of the Kit (package S). Automatic package of the Kit (package A) has a modification of polymerase – TechnoTaq MAX polymerase. It hampers the admixture of PCR components at low temperatures, thus providing a "hot-start" feature, which prevents unspecific PCR.

Real-time PCR technology is based on measurement of fluorescence at every cycle of reaction. The PCR-mix contains target-specific hydrolyzing probes bearing reporter and quencher molecules. Once

hybridized to a target sequence, the probes become activated. As a result of activation, fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The **Femoflor® *Screen* REAL-TIME PCR Detection Kit** includes following components: PCR-mixes for total bacterial load amplification, PCR-mixes for Lactobacillus spp. amplification, PCR-mixes for opportunistic flora and PCR-mixes for obligate pathogens DNA amplification.

One tube contains a PCR-mix for the amplification of human genomic DNA (sample intake control (SIC)). The SIC allows to exclude preanalytical error. If the amount of collected material is insufficient for the analysis, it is necessary to repeat sampling procedure. PCR-mix contains an internal control sample (IC) for assessment of the PCR effectiveness.

The fluorescent dyes are assigned to individual types of sequences. The Fam, Rox and Cy5 dye label are used to detect specific sequences. The Hex dye label is used to detect SIC and IC. Use of two or more distinguishable dyes allows detection of several PCR products simultaneously (see Table 1).

Defined tubes contain additional probe with Rox dye label – "Marker". It tags the strip orientation. Upon completion of run, software defines actual position of the strip (by means of "marker" position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Tube №	**Fam**	**Hex**	**Rox**	**Cy5**	**Color of the buffer**
1	Total bacterial load (TBL)[1]	IC	-	-	Blue
2	*Lactobacillus* spp.	IC	-	-	Colorless
3	*Gardnerella vaginalis/ Prevotella bivia/ Porphyromonas* spp	IC	-	-	
4	*Ureaplasma (urealyticum + parvum)*	IC	-	-	
5	*Candida* spp.	SIC	Marker	-	
6	*Mycoplasma hominis*	IC	*Mycoplasma genitalium*	-	
7	*Trichomonas vaginalis*	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	
8	Herpes simplex virus 2	IC	Cytomegalovirus	Herpes simplex virus 1	

Upon completion of the run, software of thermal cyclers performs relative quantitative analysis of total bacterial DNA, genus-specific DNA of Lactobacillus spp. and genus/species-specific DNA of each the opportunistic pathogens (or flora). The obtained value (decimal logarithm (log) of the target DNA copy concentration) does not reflect the absolute amount of the microorganisms in the biomaterial sample. This value is auxiliary for comparing the number of microorganisms.

Decimal logarithm (log) of concentration (number of copies of target DNA in 1.0 ml of DNA preparation) is determined using the threshold cycle comparison method, also called the ΔΔCt method. The advantage

[1] The term previously used is total bacterial mass (TBM).

of the ΔΔCt method is a more accurate determination of the ratio of the number of DNA targets even under conditions of suboptimal PCR efficiency without the need to build a calibration curve (Shefe J. H. Quantitative real-time RT-PCR data analysis: current concepts and the novel "gene expression's Ct difference" formula [Text] / J. H. Shefe, K. E. Lehmann, I. R. Buschmann, T. Unger, H. Funke-Kaiser // J. Mol. Med. - 2006. - Vol. 84. - P. 901–910 [DOI 10.1007/s00109-006-0097 -6]).

The calculation considers the values of the threshold cycle (Ct) and amplification efficiency. The threshold cycle is a point on the abscissa of the product accumulation graph depending on the amplification cycle number. To find the threshold cycle, the Crossing point method is used - determining the maximum of the second derivative (Cp) (Rebrikov et al., 2014). PCR efficiency is a number showing how many times the number of DNA fragments will increase in one amplification cycle.

The calculation is carried out according to the formula:

$$Log(NO_{\mathrm{a}}) = Log(E) \times (Cp - Cp_{\mathrm{a}}), \quad (1)$$

where $N0_a$ is the initial number of copies of the target DNA in 1.0 ml of the DNA preparation of the test sample;

E is PCR efficiency;

Cp is the threshold cycle for a sample containing a single DNA molecule;

Cpa - threshold cycle for the analyzed sample.

The values of E and Cp are constants, approximately equal to 2 and 45, respectively. At the maximum PCR efficiency value of 2, and in the presence of detectable DNA in the test sample, the amount of the target amplicon increases 10 times (by 1 Log) in 3.4 amplification cycles. The exact value of these parameters is indicated considering the copy number of the corresponding target for each set of primers and probes in the detection cycler software (see Annex A of to the instruction for use).

Based on the values obtained, it is possible to compare the representation of the microorganism relative to other microorganism and the total bacterial load and draw a conclusion about microbiota composition. A difference of 1 Log is 10 times the difference in concentration, a difference of 2 Log is 100 times, and so on.

Algorithm of interpretation of results is shown in chapter 10. DATA ANALYSIS.

3. CONTENT

The **Femoflor® Screen REAL-TIME PCR Detection Kit** content is represented in the Tables 2 - 3.

Table 2. The **Femoflor® Screen REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P804-S3/5EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Transparent colorless or blue liquid under waxy white fraction	3840 µL (20 µL in each tube)	24 8-tube strips
Taq-polymerase MAX solution	Transparent colorless liquid	2000 µL (500 µL in each tube)	4 tubes
Mineral oil	Transparent colorless viscous oily liquid	4.0 mL (1.0 mL in each tube)	4 tubes
Positive control	Transparent colorless liquid	160 µL	1 tube
Strip’s caps	24 8-caps		

Table 3. The **Femoflor® Screen REAL-TIME PCR Detection Kit** content, package A (automated dosing) for R1-P804-XA/4EU

Reagent	Description	Total volume	Amount
PCR-mix Stream	Transparent colorless or blue liquid	2240 µL (140 µL in each tube)	2 8-tube strips
PCR-buffer Stream-K	Transparent colorless liquid	1200 µL (600 µL in each tube)	2 tubes
TechnoTaq MAX polymerase	Transparent colorless viscous liquid	120 µL (60 µL in each tube)	2 tubes
DNA dilution buffer	Transparent colorless liquid	4 mL (1.0 mL in each tube)	4 tubes
Positive control	Transparent colorless liquid	200 µL (100 µL in each tube)	2 tubes
Strips	10 8-strips		
Strip's caps	2 8-caps		

All components are ready to use and do not require additional preparation for operation.

The **Femoflor® Screen REAL-TIME PCR Detection Kit** is used to perform 24 tests for package S and 48 tests for package A (one run of 48 samples or two runs of 24 samples), including positive and negative controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts;
- Sterile tubes containing transport medium: "DNA-Technology" made **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Physiological saline solution 0.9% NaCl (Sterile);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA PLUS** (REF P-002/2EU) or **PREP-GS PLUS** (REF P-003/2EU) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended);
- High speed centrifuge (RCF(g) no less than 16000);
- Thermostat (temperature range 50-98°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);

- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 50 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **Femoflor® Screen REAL-TIME PCR Detection Kit**, except the TechnoTaq MAX polymerase (package A), must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance. The TechnoTaq MAX polymerase (package A) must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C during the transportation. It is allowed to transport TechnoTaq MAX polymerase (package A) at temperatures from 2 °C to 8 °C for no more than 5 days.

After the first opening of the primary container the kit components should be stored under the following conditions:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period (12 months from the date of manufacture of the kit);
- PCR-mix and paraffin sealed PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period (12 months from the date of manufacture of the kit);

- TechnoTaq MAX polymerase (package A) should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period (12 months from the date of manufacture of the kit).

Keep freeze-thaw cycles of TechnoTaq MAX polymerase (package A) to a minimum, no more 5 freeze-thaw cycles.

An expired **Femoflor® Screen REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Femoflor® Screen REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Femoflor® Screen REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Avoid producing spills or aerosol. Any material being exposed to biological samples must be handled according to the local legal regulation regarding biological waste.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR

waste only in a closed form. Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **Femoflor® Screen REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from epithelial scrapes from cervical canal, posterolateral vaginal vault and urethra, depending on professional prescription.

Sampling

The sampling is held by sterile swab (or brush). The sample is then transferred to the 1.5 mL plastic tubes containing 300 µL of physiological saline solution or in the tubes containing transport media ("DNA-Technology" made **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) are recommended) or equivalent for the transportation of the sample.

General requirements

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

PCR analysis refers to direct methods of laboratory research, therefore the collection of biological material must be carried out from the site of infection localization.

Material

Professional prescription is required to localize the place of sampling (urethra, cervix or vaginal wall). The decision must be based on a patient's complaints and clinical signs, and made by the physician in charge.

Women must not perform hygiene procedures or syringing prior the sampling procedure.

To interpret results successfully and robustly, sample must contain the largest possible number of epithelial cells with minimum amounts of mucus and blood. Inappropriate sampling may result in doubtful results and sample collection may need to be repeated.

The features of the vaginal sampling:

The sample must be taken prior to physical inspection. Speculum can be treated by warm sterile saline solution before the procedure. Antiseptics must not be used for speculum treatment. The sample must be taken from the lateral or posterolateral vaginal wall.

From virginal women, sample must be taken from the entrance of vagina, or in special cases from the posterior vaginal wall through the hymenal rings.

The features of the urethral sampling:

Patient must not urinate within 1.5-2 hours prior to sampling procedure.

The external urethral orifice must be treated with a swab moistened with sterile physiological saline solution just prior to the sampling procedure.

In the case of purulent discharge, the sample must be taken 15-20 minutes after urinating. In the absence of discharge, it is necessary to massage urethra with sampling swab or brush.

Carefully insert the swab into the woman's urethra to a depth of 1-1.5 cm. A child's sample must be taken from the external urethral orifice.

The features of the cervical sampling:

Remove mucus with a swab prior to sampling, and treat the cervix with sterile physiological saline solution.

Carefully insert sampling swab into the cervix to a depth of 0.5-1.5 cm.

Avoid contact with vaginal wall when removing the swab.

Order of sampling in the tubes containing transport media:

1. Open the tube
2. Scrape epithelial cells from the corresponding biotope (i.e., vagina, urethra, cervix) with a sterile swab.
3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab. Use a new swab if you need to repeat sampling or to take sample from another biotope.
5. Close the tube tightly and mark it.

Transportation and storage of the samples

ATTENTION! Overall time from the sample intake until analysis must not exceed 24 hours.

Samples may be transported and stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

NOTE - The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-NA PLUS**, **PREP-GS PLUS** and **PREP-MB RAPID** extraction kits user manuals.

The presence of PCR inhibitors may cause false-negative results.

Interfering substances include (but are not limited to) the following:

- The presence of mucus in cervical samples may inhibit PCR and cause false-negative results. Use a sponge or large swab to remove cervical secretions;
- The traces of blood with hemoglobin can affect the test performance;
- The heparin is shown to be a strong inhibitor of the PCR;
- Data about possible inhibiting effects of drugs is not available.

8. PROCEDURE

DNA extracting from biological material.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA PLUS**, **PREP-GS PLUS** and **PREP-MB RAPID** extraction kits are recommended.

NOTE - A DNA extraction kit is not included in the **Femoflor® Screen REAL-TIME PCR Detection Kit**.

If the sample is taken in a tube with sterile physiological saline solution, the "DNA-Technology" **PREP-NA PLUS DNA/RNA Extraction Kit** (REF P-002/2EU) or **PREP-GS PLUS DNA Extraction Kit** (REF P-003/2EU) are recommended for DNA extraction. If the sample is taken in a tube with the **PREP-RAPID DNA Extraction Kit** (REF P-001/1EU) reagent, **PREP-NA PLUS DNA/RNA Extraction Kit** is recommended for DNA extraction.

Enquire with customer service about compatibility of third-party DNA extraction kits with **Femoflor® Screen REAL-TIME PCR Detection Kit**.

ATTENTION! Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control sample in volumes as indicated.

8.1 Assay procedure for package S:

ATTENTION! The reagents and tubes should be kept away from direct sun light.

ATTENTION! Please, make sure that you use corresponding strips and caps for strips from the same kit, while using the kit. Do not use caps for strips from other kits!

8.1.1. Mark strips with paraffin sealed PCR-mix for test samples, positive control (C+) and negative control (C-).

One strip is designed for one sample assay.

Example: to test 2 samples, mark 1 strip for each sample, 1 strip for "C-" and 1 strip for "C+". The resulting number of strips is 4.

8.1.2. Vortex the Taq-polymerase MAX solution for 3-5 seconds, then spin for 1-3 seconds.

8.1.3. Add 10 µL of Taq-polymerase MAX solution into each tube. Avoid paraffin layer break.

8.1.4. Add one drop (~20 µL) of mineral oil into each tube. Close tubes.

ATTENTION! In case of using **PREP-GS PLUS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

ATTENTION! In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

ATTENTION! Open the strip, add DNA sample, then close the strip before proceeding to the next DNA sample to prevent contamination. Close strips tightly. Use filter tips.

8.1.5. Add 5 µL of DNA sample into corresponding strip tubes. Do not add DNA into the "C-", "C+" strip tubes. Avoid paraffin layer break.

8.1.6. Add 5 µL of C- which passed whole DNA extraction procedure into "C-". Add 5 µL of C+ into corresponding strip tubes. Avoid paraffin layer break.

8.1.7. Spin the tubes for 1-3 seconds.

8.1.8. Set the strip into the thermal cycler. Try to place tubes in the center of the thermoblock.

8.1.9. Launch the operating software for DT instrument[2]. Add corresponding test[3], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.1.8) and run PCR. See Table 4.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10[1]		…	Holding		Holding

√ - optical measurement
[1] – holding at 25°C is allowed

Note: The test for the DTprime, DTlite devices can be created by entering the parameters in accordance with Annex A, or this test is provided by the reagent kit manufacturer.

8.2 Assay procedure for package A (using DTstream):

8.2.1. Spin the strip tubes with PCR-mix Stream for 1-3 seconds.

8.2.2. Vortex the tubes with PCR-buffer Stream-K and TechnoTaq MAX polymerase for 3-5 seconds and spin for 1-3 seconds to collect drops.

ATTENTION! TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.2.3. Prepare a mixture of PCR-buffer Stream-K with TechnoTaq MAX polymerase. Add PCR-buffer Stream-K from one tube to another tube with PCR-buffer Stream-K, then add TechnoTaq MAX polymerase from both of tubes with TechnoTaq MAX polymerase. Close the tube.

8.2.4. Vortex the tube with prepared mixture for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

ATTENTION! The mixture of PCR-buffer Stream-K and TechnoTaq MAX polymerase must be prepared just prior to use.

8.2.5. Add 140 µL of the mixture of PCR-buffer Stream-K with TechnoTaq MAX polymerase to each tube in strip.

8.2.6. Mark the empty strips included in the Kit.

8.2.7. Add 100 µL of DNA dilution buffer to each strip tube.

[2] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[3] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.2.8. Add 40 µL of samples, “C+” and “C-” to corresponding strip tubes with of DNA dilution buffer.

8.2.9. Set strip tubes with PCR-mix Stream, PCR-buffer Stream-K and TechnoTaq MAX polymerase mixture, diluted samples, positive control and negative control, empty strip tubes and microplate for PCR to the DTstream and dispense the components according to the instruction manual.

8.2.10. After completion of the program on the DTstream, set gently, without shaking, the microplate to the DTpack.

8.2.11. Carry out the procedure of sealing the microplate by thermal film in accordance with the instructions to the DTpack.

8.2.12. Spin the microplate at RCF(g) 1000 for 30 seconds.

8.2.13. Set the microplate to the Thermal Cycler.

8.2.14. Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.2.13) and run PCR.

9. CONTROLS

The **Femoflor® Screen REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the specific genomes. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance. To reveal possible contamination a negative control is required.

ATTENTION! A negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

The test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not considered;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does not express growing fluorescence of the specific product or positive result, it is required to repeat the whole testing run. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

Registration of the PCR results is performed in automatic mode. The identificator of sample, test name and result for each target (diagram allowing a relative comparison of normal flora and opportunistic pathogens in each sample) will be displayed. A qualitative analysis will be performed for pathogens.

In the samples containing specific product DNA (specific product), the PCR program detects a positive result. In this case, the amplification result of the internal control is not considered. The detecting thermal

[4] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[5] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

cycler registers the expressed growing fluorescence in specific product, the amplification result of the internal control is not considered.

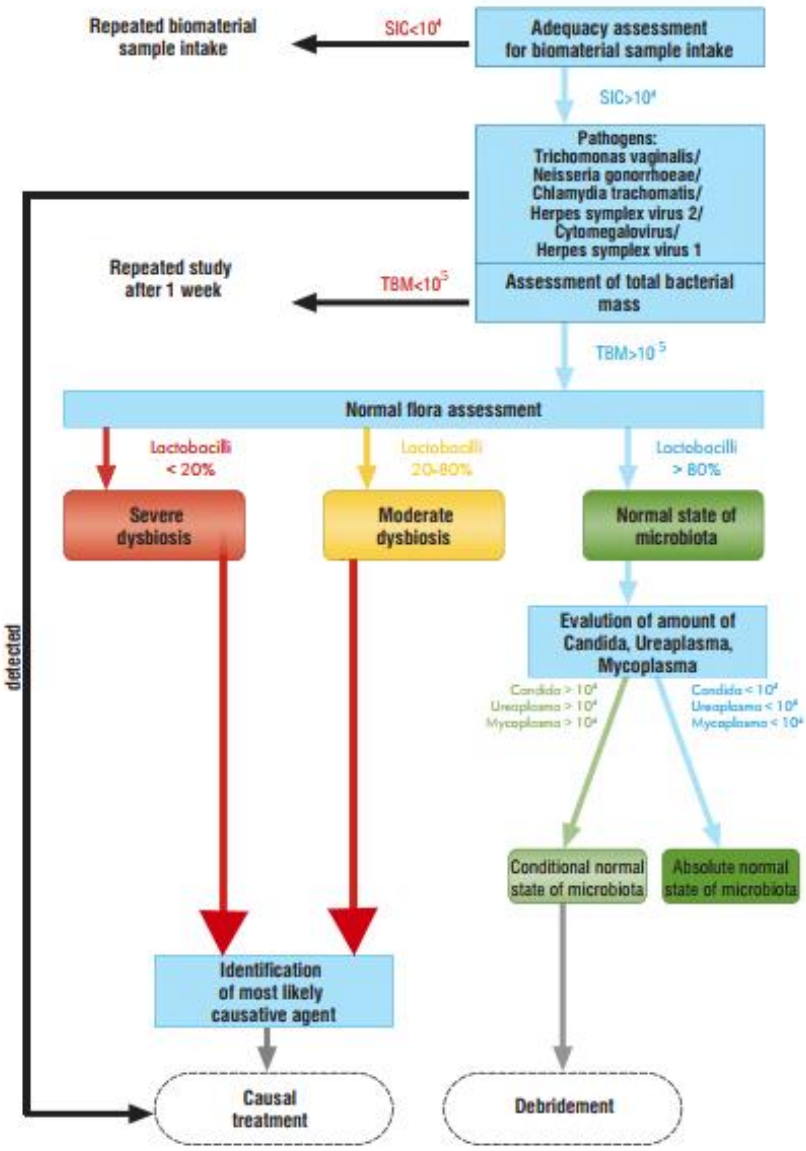
In the samples free of specific product DNA, the PCR program detects a negative result of the specific product and positive result of the internal control. The detecting thermal cycler registers the expressed growing fluorescence in the internal control and its absence in the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

When SIC (Sample Intake Control) value is lower than 4.0, it should be considered as insufficient amount human DNA in sample and the sampling procedure must be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual).



11. SPECIFICATIONS

a. The analytical **specificity** of the **Femoflor® Screen REAL-TIME PCR Detection Kit** was assessed by bioinformatic analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with specific product DNA are to be registered positive for specific product (a fragment of the specific product genome). The samples free of specific product DNA are to be registered negative for specific product and positive for internal control.

The list of microorganisms identified by the kit is presented in Table 5.

Table 5. The list of microorganisms identified by the **Femoflor® Screen REAL-TIME PCR Detection Kit**.

№	Detection channels			
	Fam	Hex	Rox	Cy5
1	Total bacterial load (TBL)	IC	-	-
2	Lactobacillus spp.	IC	-	-
3	*Gardnerella vaginalis*/ *Prevotella bivia*/ Porphyromonas spp.	IC	-	-
4	Ureaplasma (urealyticum + parvum)	IC	-	-
5	Candida spp.	SIC	Marker	-
6	*Mycoplasma hominis*	IC	*Mycoplasma genitalium*	-
7	*Trichomonas vaginalis*	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*
8	Herpes simplex virus 2	IC	Cytomegalovirus	Herpes simplex virus 1

b. In a determination of analytical **sensitivity,** the **Femoflor® Screen REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 5 or more copies of purified pathogens DNA per PCR reaction. The copy number of the pathogens was determined by Poisson analysis.

The **Femoflor® Screen REAL-TIME PCR Detection Kit** detect one CFU of the pathogen per PCR reaction. This analytical sensitivity was assessed by serial dilution of pathogens infected cultures in culture transport media. Samples of each dilution were processed and tested by the standard Kit procedure. Each of the replicates containing 1 CFU per amplification reaction gave a strong positive signal.

The analytical sensitivity is no more than 10,000 copies/mL, except *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Cytomegalovirus, Herpes simplex virus 1, Herpes simplex virus 2.

For *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Cytomegalovirus, Herpes simplex virus 1, Herpes simplex virus 2, the analytical sensitivity is 2000 copies/mL.

c. Sample Intake Control

During amplification of biological samples containing human genomic DNA the real-time PCR instrument should record the exponential growth of the fluorescence level in the corresponding tube.

During amplification of biological samples that do not contain the human genomic DNA the real-time PCR instrument should record the absence of exponential growth of the fluorescence level in the corresponding tube.

d. Diagnostic characteristics

Characteristic	Diagnostic Sensitivity (CI 95%)	Diagnostic Specificity (CI 95%)
Normoflora – Lactobacillus spp.	99.71% (98.41 - 99.99%)	97.73% (87.98 - 99.94%)
Gardnerella vaginalis/ Prevotella bivia /Porphyromonas spp.	100.00% (98.37 - 100.00%)	99.40% (96.73 - 99.98%)
Mycoplasma hominis	98.61% (92.50 - 99.96%)	100.00% (98.86 - 100.00%)
Mycoplasma genitalium	100.00% (88.06 - 100.00%)	100.00% (98.99 - 100.00)
Ureaplasma (urealyticum + parvum)	100.00% (96.92 - 100.00%)	100.00% (98.67 - 100.00%)
Candida spp.	100.00% (97.47 - 100.00%)	98.80% (96.52 - 99.75%)
Trichomonas vaginalis	100.00% (88.78 - 100.00%)	99.72% (98.47 - 99.99%)
Neisseria gonorrhoeae	100.00% (88.78 - 100.00%)	100.00% (98.99 - 100.00%)
Chlamydia trachomatis	100.00% (90.00 - 100.00%)	100.00% (98.97 - 100.00%)
Herpes simplex virus 1	96.30% (81.03 - 99.91%)	100.00% (99.00 - 100.00%)
Cytomegalovirus	100.00% (87.66 - 100.00%)	100.00% (98.99 - 100.00%)
Herpes simplex virus 2	100.00% (86.77 - 100.00%)	100.00% (99.00 - 100.00%)

NOTE - The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA PLUS** REF P-002/1EU, **PREP-GS PLUS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU kits.

12. TROUBLESHOOTING

Table 6. Troubleshooting

	Result	**Possible cause**	**Solution**
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Contact our customer service with quality issues of **Femoflor® Screen REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
	Caution		Do not use reuse
EC REP	Authorized representative in the European Community		

Annex A

Run parameters to be entered into the Thermal Cyclers software

1) "Type of analysis" - select "Biocenosis" or «Multiplex Q+»;

2) Specify «the amount of the tubes» in the test - 8;

3) Specify «PCR-mix volume»:

- for «Package S»: 35 µL;

- for «Package A»: 12 µL.

4) In the "Amplification program" window, enter the following parameters:

Package S. The PCR program for DTlite and DTprime (*M*) Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10[1]	…	…	…		Holding
√- optical measurement [1] – holding at 25°C is allowed						

Package A. The PCR program for DTprime (*X*) Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	05	15		Cycle
	94	0	05			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	10[1]	…	…	…		Holding
√- optical measurement [1] – holding at 25°C is allowed						

5) "Detection channels", enter the following parameters

Detection channels of amplification products for **Femoflor® Screen Screen REAL-TIME PCR Detection Kit**

№	Detection channels			
	Fam	Hex	Rox	Cy5
1	Total bacterial load (TBL)	IC	-	-
2	Lactobacillus spp.	IC	-	-
3	*Gardnerella vaginalis*/ *Prevotella bivia*/ Porphyromonas spp.	IC	-	-
4	Ureaplasma (urealyticum + parvum)	IC	-	-
5	Candida spp.	SIC	Marker	-
6	*Mycoplasma hominis*	IC	*Mycoplasma genitalium*	-
7	*Trichomonas vaginalis*	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*
8	Herpes simplex virus 2	IC	Cytomegalovirus	Herpes simplex virus 1

6) "Amplification parameters", in the «Additional settings» window, enter the coefficients and the minimum values of the concentration logarithm for each analyte

Femoflor® Screen REAL-TIME PCR Detection Kit

№	Channel	Name of research	N-i	E_i	min_Result	%_Flam
1(1)	Fam	TBL	44.8	3.40	3.0	0
1(2)	Hex	IC	45.0	3.40	0.0	0
2(1)	Fam	*Lactobacillus*_spp.	45.3	3.40	3.0	0
2(2)	Hex	IC	45.0	3.40	0.0	0
3(1)	Fam	*Gardnerella_vaginalis+Prevotella_bivia+Porphyromonas*_spp.	45.0	3.40	3.0	0
3(2)	Hex	IC	45.0	3.40	0.0	0
4(1)	Fam	*Ureaplasma*_ spp.	45.0	3.40	0.0	0
4(2)	Hex	IC	45.0	3.40	0.0	0
5(1)	Fam	Candida_spp.	45.0	3.40	3.0	0
5(2)	Hex	SIC	46.0	3.51	3.0	0
5(3)	Rox	Marker	45.0	3.40	0.0	0
6(1)	Fam	*Mycoplasma_hominis*	45.0	3.40	0.0	0
6(2)	Hex	IC	45.0	3.40	0.0	0
6(3)	Rox	*Mycoplasma_genitalium*	45.0	3.40	0.0	0
7(1)	Fam	*Trichomonas_vaginalis*	45.0	3.40	0.0	0
7(2)	Hex	IC	45.0	3.40	0.0	0
7(3)	Rox	*Neisseria_gonorrhoeae*	45.0	3.40	0.0	0
7(4)	Cy5	*Chlamydia_trachomatis*	45.0	3.40	0.0	0
8(1)	Fam	HSV-2	45.0	3.40	0.0	0
8(2)	Hex	IC	45.0	3.40	0.0	0
8(3)	Rox	CMV	45.0	3.40	0.0	0
8(4)	Cy5	HSV-1	45.0	3.40	0.0	0