DNA-TECHNOLOGY

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For research use only

FEMOFLOR®DeltaScreen
REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P816-S3/5ER
R1-P816-XA/4ER
R1-P817-XA/4ER

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1. INTENDED USE

FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit is an *in vitro* Nucleic Acid Test (NAT). **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** is designed for quantitative detection of DNA of normal microbiota representatives *Lactobacillus iners* and *Lactobacillus* non-*iners*, opportunistic microorganisms *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Candida* spp*., Candida albicans, Gardnerella vaginalis, Fannyhessea vaginae, Mobiluncus* spp*., Staphylococcus* spp*., Streptococcus* spp*., Enterobacteriaceae, Enterococcus* spp*., Haemophilus* spp., qualitative and quantitative detection of DNA of pathogenic microorganisms *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis,* HSV1, HSV2, CMV in biomaterial from female reproductive tract (epithelial swab from the mucous membrane of vagina and cervical canal) by real-time PCR.

Population and demographic aspects: the reagent kit is designed for examination of post-pubertal women.

Potential users: qualified personnel trained in molecular research methods.

Apply the kit only as directed in the present instruction for use.

2. METHOD

Method: multiplex polymerase chain reaction (PCR) with results detection in real-time; quantitative and qualitative multiplex analysis.

The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent elongation of polynucleotide chains from these primers with Taq polymerase.

To increase the sensitivity and specificity of the amplification reaction, hot start is used. For package S, hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The PCR starts only when paraffin is melted. “Hot” start for packages A and A-TL is provided by blocking the activity of Taq polymerase with antibodies. The enzyme activates only after preheating the reaction mixture at 94°C. This prevents the nonspecific annealing of primers on the DNA target at low temperatures during the initial heating of the test tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the PCR mix. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of hydrolyzed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

The reagent kit includes PCR mixes specific for lactobacilli, pathogenic and opportunistic microorganisms, DNA of all bacteria (Total Bacterial Load – TBL) and human genomic DNA (HGD).

PCR mixes (except for tubes No. 1 and tube No. 5) include internal control (IC) intended for quality assessment of PCR in each tube.

A marker oligonucleotide with a fluorescent dye Cy5.5 is added into tube No.3 to control the position of strips in the thermoblock. After amplification, the software compares the marker position specified by the operator with its real position, and warns the operator in case of incorrect strip positioning. In this case the operator needs to verify the position of the strip in the thermoblock (the first tube is marked with a blue buffer) and correct the tube IDs in the protocol.

The DNA probes used for detection of specific analytes include fluorescent tags: Fam, Hex, Rox, Cy5, Cy5.5. The DNA probe used for detection of the amplification product of internal control (IC) includes fluorescent dye Hex.

The use of several fluorescent dyes allows to reduce the number of tubes and biomaterial required for the assay by simultaneously registering the results of multiple amplification reactions.

Table 1 shows the detected microorganisms, color coding, and detection channels for amplification products.

Table 1. Detection channels of amplification products for **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit**

Tube No.	**Detection channels**					**PCR mix color marking**
	Fam	Hex	Rox	Cy5	Cy5.5	
1	*Lactobacillus* non-*iners*	*Lactobacillus iners*	*Fannyhessea vaginae*	Total bacterial load (TBL)	*Gardnerella vaginalis*	Blue
2	*Staphylococcus* spp.	IC	*Streptococcus* spp.	-	-	Colorless or pink
3	*Mobiluncus* spp.	IC	-	*Haemophilus* spp.	Marker	
4	-	IC	*Enterobacteriaceae*	*Enterococcus* spp.	-	
5	*Mycoplasma hominis*	*Ureaplasma urealyticum*	*Ureaplasma parvum*	Human genomic DNA	-	
6	*Candida* spp.	IC	*Candida albicans*	-	-	
7	*Trichomonas vaginalis*	IC	*Mycoplasma genitalium*	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*	
8	HSV2	IC	CMV	HSV1	-	

The automatic analysis is available on DNA-Technology instruments: DTlite, DTprime or DTprime II real-time thermal cyclers (see https://www.dna-technology.com for available supply options). The current software version is available for download at https://www.dna-technology.com/software.

The indicator cycle number and PCR-efficiency are used to calculate the amount of target analyte. To determine the indicator cycle, the crossing point method (Cp) is used. The Cp method is based on the calculation of the maximum of the second derivative of a fluorescent graph. PCR efficiency is an indicator of the increase in the number of DNA fragments within one amplification cycle.

The decimal logarithm (Lg) of initial DNA concentration in a sample (number of DNA target copies per 1.0 mL of DNA sample) is calculated using indicator cycle comparison method, also known as ΔΔCt method. The ΔΔCt method makes it possible to determine the initial amount of target DNA in a sample without the need to construct a calibration curve.

Calculation is carried out according to the formula:

$$Log(NO_a) = Log(E) \times (Cp - Cp_a), \qquad (1)$$

where $N0_a$ is the initial number of target DNA copies in 1.0 mL of DNA sample;

E – PCR efficiency;

Cp – indicator cycle for the sample containing a single DNA molecule;

Cp_a – indicator cycle for the test sample.

The assay consists of the following steps: DNA extraction (DNA sample preparation) and DNA PCR amplification with detection of the results in real time using **FEMOFLOR®DeltaScreen** reagent kit.

3. CONTENTS

FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit comes in a standard package **S** as well as in automated dosing packages **A**, **A-TL**.

The content of the reagent kit is presented in Tables 2-4.

Table 2. **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** content, package S (strips) for R1-P816-S3/5ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless, pink or blue transparent liquid under waxy white fraction	3.84 mL (20 µL in each)	tubes, 24 strips of 8
Taq polymerase solution	Colorless transparent liquid	2.0 mL (1.0 mL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	4.0 mL (1.0 mL in each)	4 tubes
Positive control[1]	Colorless transparent liquid	160 µL	1 tube
Strip caps	24 strips of 8		

Table 3. **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** content, package A for R1-P816-XA/4ER

Reagent	Description	Total volume	Amount
PCR mix Stream	Colorless, pink or blue transparent liquid	1.92 mL (120 µL in each)	tubes, 2 strips of 8
PCR buffer Stream	Colorless transparent liquid	1.2 mL (600 µL in each)	2 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	120 µL (60 µL in each)	2 tubes
NA dilution buffer	Colorless transparent liquid	4.0 mL (1.0 mL in each)	4 tubes
Positive control[1]	Colorless transparent liquid	200 µL (100 µL in each)	2 tubes
Tubes	10 strips of 8		

Table 4. **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** content, package A-TL for R1-P817-XA/4ER

Reagent	Description	Total volume	Amount
PCR mix Stream	Colorless, pink or blue transparent liquid	1.92 mL (120 µL in each)	tubes, 2 strips of 8
TL buffer	Colorless transparent liquid	1.43 mL (715 µL in each)	2 tubes
Polymerase TL-65	White or beige dry substance	2 tubes (lyophilized)	
NA dilution buffer	Colorless transparent liquid	4.0 mL (1.0 mL in each)	4 tubes
Positive control[1]	Colorless transparent liquid	200 µL (100 µL in each)	2 tubes
Tubes	10 strips of 8		

All components are ready for use.

FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit in package S is designed for 24 tests (no more than 8 runs), which includes analysis of test samples, negative and positive controls.

[1] - Positive control is marked as "C+" for all packages.

FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit in packages A, A-TL is designed for 48 tests (one run of 48 samples or two runs of 24 samples each), which includes analysis of test samples, negative and positive controls.

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

Table 5. Equipment, reagents and consumables

Equipment, reagents and consumables	Package S	Package A, Package A-TL
UV PCR cabinet	yes	yes
Real-time PCR thermal cycler	yes[1]	yes[2]
Vortex mixer	yes	yes
Vortex rotor for 0.2 mL strip tubes	yes	yes
Refrigerator or a cooling chamber	yes	yes
Freezer	no	yes
Tube rack for 0.2 mL strip tubes	yes	no
Tube rack for 1.5 mL tubes	yes	yes
Single channel pipettes (dispensers covering 2.0-20, 20-200, 200-1,000 µL volume range)	yes	yes
RNase- and DNase-free aerosol barrier pipette tips (volumes 10 µL, 20 µL, 200 µL, 1000 µL)	yes	yes
Pipette rack	yes	yes
RNase- and DNase-free 1.5 mL microfuge tubes with caps	yes	no
Powder-free surgical gloves	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes
Automated Liquid Handling station	no	yes[3]
RNase and DNase free filter 200 µL pipette tips for DTstream or similar ones recommended for DTstream	no	yes
Microplate Heat Sealer	no	yes[4]
Centrifuge with microplate adapter, RCF(g) ≥100	no	yes
Polymer thermal sealing film for microplates	no	yes
384-well PCR microplate	no	yes
Transport medium[5]: - **STOR-F** (DNA-Technology, Russia); - **STOR-M**[6] (DNA-Technology, Russia). Transport media for liquid cytology: - **PreservCyt®**, Hologic Inc., USA; - **BD SurePath™ Liquid-Based Pap Test**, Becton, Dickinson and Company, USA; - **EASYPREP**, YD Diagnostics, South Korea; - **CellPrep**, CP Biodyne, South Korea; - **Cell Preservative Solution**, Hunan Lituo Biotechnology Co.Ltd, China.		
- Medical swabs, types A, A1 Self-sampling devices: - **Qvintip®** self-sampling device, Aprovix AB, Sweden; - **FLOQSwabs®** swab system, Copan Italia S.p.A., Italy.		
Physiological saline solution 0.9% NaCl (sterile)		
NA extraction kit: - **PREP-NA PLUS**; - **PREP-GS PLUS**; - **PREP-MB MAX**; - **PREP-MB-RAPID II**.		
Notes: [1] – DTprime 5M*, DTprime II 5M*, DTlite 5S* (DNA-Technology R&P, LLC) [2] – DTprime 5X* or DTprime II 5X* (DNA-Technology R&P, LLC)		

Equipment, reagents and consumables	Package S	Package A, Package A-TL
[3] – DTstream *M4 (DNA-Technology R&P, LLC) [4] – DTpack plate sealing (DNA-Technology R&P, LLC) or similar [5] – it is allowed to use any transport media designed for storage and transport of PCR biomaterial [6] – not recommended for use together with the **PREP-MB-RAPID II DNA/RNA Extraction Kit**		

The following detecting thermal cyclers are validated for work with the **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit**:

- DTprime in DTprime 5M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime;
- DTprime II in DTprime II 5M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II;
- DTprime in DTprime 5X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime 5X* (only for packages A, A-TL);
- DTprime II in DTprime II 5X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II 5X* (only for packages A, A-TL);
- DTlite in DTlite 5S* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTlite.

For detection thermal cyclers not mentioned above please contact the manufacturer of the reagent kit.

Software:

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

Supported OS: Windows 7 and above.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of manufacture.

5.1. Storage conditions

5.1.1. Package S

- All components of **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored away from light over the storage period.

5.1.2. Package A

- All components of **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit**, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix Stream must be stored away from light over the storage period.
- The TechnoTaq MAX polymerase must be stored in a freezer at temperatures ranging from minus 22°C to minus 18°C over the storage period.

5.1.3. Package A-TL

- All components of the **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix Stream must be stored away from light over the storage period.
- Polymerase TL-65 must be stored with desiccant in a sealed foil bag over the storage period.

WARNING! The excessive temperature and light can be detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

5.2.1. **Package S**

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

5.2.2. **Package A**

- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes up to 25°C for no longer than 5 days.

5.2.3. **Package A-TL**

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. **Package S**

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.

5.3.2. **Package A**

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix Stream must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures ranging from minus 22°C to minus 18°C over the storage period.

5.3.3. **Package A-TL**

- All components of the must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix Stream must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.
- Polymerase TL-65 must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C with desiccant in a sealed foil bag over the storage period.

WARNING! Kits stored with violation of storage conditions must not be used.

An expired **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** must not be used.

We strongly recommend following the current instructions for use in order to obtain accurate and reliable results.

The manufacturer guarantees the conformity of **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** to the technical documentation if the storage, transportation and handling requirements are fulfilled.

6. WARNINGS AND PRECAUTIONS

- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or unreliable results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.
- Avoid any direct contact with the biological samples, reagents and materials used to carry out the assay. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- 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 only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips. The tips employed must be sterile, free from DNases and RNases and 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 to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as if infectious[2, 3]. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 minutes or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must 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 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. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 minutes before and after the assay. All

[2] - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

[3] - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

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

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

Do not use the kit:

- If the transportation and storage conditions have been violated;
- If the appearance of the reagents does not correspond to the product documentation;
- If the packaging of the kit components is breached;
- After the expiry date.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

7. SAMPLES

FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit is designed to detect DNA extracted from epithelial swabs from mucous membrane of vagina and cervical canal.

7.1. General requirements

PCR analysis is a direct diagnostic method, therefore, the collection of biological material must be carried out from the site of infection localization. The biomaterial type is selected by the physician based on the collected anamnesis and clinical picture of the disease.

The quality of sample collection, sample storage, transport and pretreatment is crucial for obtaining correct results. Incorrect sample collection may lead to unreliable results and, therefore, to the need to repeat the sample collection procedure.

If biomaterial from several biotopes is required, repeat the procedure using a new swab for each biotope.

Use RNase- and DNase-free aerosol barrier pipette during biomaterial preparation and NA extraction.

To prevent contamination, only open one tube cap at a time and close it before proceeding to the next tube.

7.2. Interfering substances

The presence of PCR inhibitors in a sample may cause unreliable (invalid) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

Potential PCR inhibitors and their maximum concentrations that may be present in the sample which may inhibit PCR are shown in Table 6.

Presence of possible inhibition was evaluated be adding potential interfering substances into biomaterial samples in concentrations shown in Table 6. Then DNA extraction and real-time PCR using **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** were performed. As a result, potential PCR inhibitors showed no influence on PCR performed using **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit**.

Table 6. Interfering substances used for evaluation of possible PCR inhibition

Biomaterial	Interfering substance	Active substance	Type of interfering substance	Substance concentration in DNA preparation
Swabs/scrapes from vagina, cervical canal	Whole blood	Hemoglobin	Endogenous	0,1% v/v (0.35 mg/mL)
	Mucus	Mucin		10%
	Miramistin®, 0.01% solution	Benzyldimethyl [3-(myristoylamino) propyl] ammonium chloride monohydrate	Exogenous – local antiseptic	10% (0.001%)
	Chlorhexidine bigluconate 0.05% solution	Chlorhexidine bigluconate		10% (0.005%)
	Isopropyl alcohol	Isopropyl alcohol	Exogenous – DNA extraction kits' component	10% (100 µL/mL)
	Methyl acetate	Methyl acetate		10% (100 µL/mL)

7.3. Sample collection and preparation

WARNING! Sample pretreatment may be required before DNA extraction.

Sample collection is performed using specialized medical devices in accordance with the procedure outlined below. Additionally, consult the instructions for use for NA extraction reagent kit and self-sampling device before collecting the sample.

Table 7. Preparation for biomaterial collection

BEFORE THE TEST, IT IS NOT ALLOWED TO	IF THE GUIDELINES ARE NOT FOLLOWED, biomaterial can be collected no sooner than
Perform syringing and vaginal douching with antiseptics	24 hours
Use tampons	
Use PCR-inhibiting substances, such as ultrasound gel, heparin, chlorhexidine and other chlorine-containing medicinal products	
Undergo transvaginal ultrasound examination	
Engage in protected sexual contacts	
Undergo colposcopy	48 hours
Engage in unprotected sexual contacts	72 hours
Take and/or use antibacterial medications	2 weeks FEMOFLOR®DeltaScreen can be prescribed to selected examinees on antibiotic therapy, for instance, for therapy monitoring. However, the test results should be interpreted with care by the specialist based on the examinee's medical history, as antibiotics can influence the outcome of the test.
Take and/or use probiotics	

WARNING! Before obtaining an epithelial cell swab from the posterior vaginal vault and cervical canal, the free-flowing secretion should be removed with a sterile cotton swab.

7.3.1 Vaginal sampling

The sample must be taken prior to physical examination. Speculum can be treated with warm water before the procedure. Antiseptics must not be used for speculum treatment. The sample must be taken from the lateral or posterolateral vaginal wall.

For virginal women, the sample must be taken from vestibular mucous membrane, or, in special cases, from the posterior vaginal wall through the hymenal rings.

7.3.2 Cervical sampling

Remove mucus with a cotton swab prior to sampling and treat the cervix with sterile physiological saline solution. Carefully insert a sampling swab into the cervix to a depth of 0.5-1.5 cm. Avoid contact with the vaginal wall when removing the swab.

7.3 Transport and storage of the samples

Transport and storage conditions for epithelial swabs from the mucous membrane of the cervical canal and vagina are outlined in the instructions for use to the recommended NA extraction reagent kits or transport media used for transport and storage of samples.

7.4 Biomaterial preparation for DNA extraction

Preparation of epithelial swabs from the mucous membrane of the cervical canal, vagina is carried out in accordance with the instructions for use for the NA extraction reagent kits and for transport media.

For samples taken into BD SurePath™ Liquid-Based Pap Test and CellPrep media, pretreatment is required. It is recommended to use Kit for sample pretreatment while processing of nucleic acid (**PREP-PK**) manufactured by DNA-Technology Research & Production, LLC, according to instructions for use.

For samples taken into PreservCyt, EASYPREP and Cell Preservative Solution media:

1. Thoroughly mix the contents of the vial with the sample in the transport medium (solution) by shaking the vial intensively.
2. Transfer 1.0 mL of material into a disposable 1.5 mL plastic tube. Close the tube tightly.
3. Centrifuge the tube at RCF(g) 12,000-16,000 for 3 minutes.
4. Carefully remove as much supernatant as possible without disturbing the pellet.
5. Add **STOR-F** transport medium or sterile saline solution to the precipitate in the volume specified in the instructions for the DNA extraction reagent kit and resuspend the precipitate by pipetting.

When using self-sampling devices, the probe is transferred into a tube with transport medium intended for the transport and storage of biomaterial samples for PCR assay. Further preparation is carried out in accordance with the instructions for use for NA extraction reagent kits or the instructions for use for the transport medium used.

8. PROCEDURE

DNA extraction from biological material

For DNA extraction from epithelial swabs from mucous membranes of vagina and cervical canal it is recommended to use the authorized NA extraction reagents kits compatible with the biomaterial, for example **PREP-NA PLUS**, **PREP-GS PLUS**, **PREP-MB-RAPID II**, **PREP-MB MAX**.

The minimal eluate volume for the reagent kits mentioned above is 300 µL.

DNA extraction is performed in accordance with the instructions for use of the corresponding reagent kit; for **PREP-NA PLUS** extraction kit short method of extraction can be used.

WARNING! Regardless of the DNA extraction kit used, the negative control must go through all stages of DNA extraction. Physiological saline solution can also be used as a negative control in volumes specified in the instruction for use for the corresponding DNA extraction kit.

8.1. PCR package S

WARNING!

- The reagents and tubes must be kept away from direct sunlight.
- Closely monitor the completeness of the strips and caps for them. Do not use strip caps from other kits!

8.1.1 Mark one strip with paraffin-sealed PCR mix for each test sample, positive (“C+”) and negative (“C-”) controls.

Note. One strip is used for one sample.

Example: to test 4 samples, mark 4 strips for test samples; one strip for “C-” and one strip for “C+”. The total number of strips is 6.

8.1.2 Shake the tube with Taq polymerase solution on vortex for 3-5 seconds and centrifuge on vortex for 1-3 seconds.

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

8.1.4 Add one drop (~20 µL) of mineral oil into each tube of the strip. Cover the strips loosely with caps.

8.1.5 Shake the tubes with “C+” for 3-5 seconds and centrifuge on vortex for 1-3 seconds.

WARNING!

- Before adding DNA and negative controls to the test tubes with the reaction mixture follow the DNA preparation guidelines outlined in the instruction for use for the NA extraction kit.
- For **PREP-NA PLUS** and **PREP-GS PLUS** (only in case the precipitate with the extracted DNA was transferred to new tubes) NA extraction kits: shake the tubes with DNA sample and negative control on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.
- For **PREP-MB MAX DNA Extraction Kit**, centrifuge the tubes with the DNA sample and negative control on vortex for 1-3 seconds (do not shake!), then place the tubes into magnetic rack. If after extraction the precipitate with the extracted DNA was transferred to new tubes, shake the tubes with the DNA sample and negative control on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.
- Before introducing DNA, only open the caps of the strips that are currently in use and close them before proceeding to the next one to prevent contamination. Close the strips tightly. Use aerosol barrier pipette tips.

8.1.6 Add 5.0 µL of DNA sample into each tube of the corresponding labeled strips. Do not add DNA into the “C+”, “C-” strips. Avoid paraffin layer break. Close the strips tightly.

8.1.7 Add 5.0 µL of the negative control (C-) (the one that underwent the whole DNA extraction procedure) into each tube of the corresponding labeled strips. Avoid paraffin layer break. Close the strip tightly.

8.1.8 Add 5.0 µL of positive control (C+) into each tube of the corresponding labeled strips. Avoid paraffin layer break. Close the strip tightly.

8.1.9 Centrifuge all strips on vortex for 1-3 seconds.

8.1.10 Place all strips into the real-time PCR thermal cycler.

8.1.11 Launch the operating software for DT instrument[4]. Add the corresponding test[5], specify the number and IDs of the samples, positive and negative controls. Specify the position of the strips in the thermoblock and run PCR. Before the run, compare your selected program to the values provided in Table 8.

Table 8. PCR program for DTlite, DTprime and DTprime II thermal cyclers (package S)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Step type
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	25*	…	…	Temperature hold		Temperature hold
*temperature hold at 10°C is allowed						

8.2 PCR packages A, A-TL using DTstream (only for DTprime 5X* and DTprime II 5X* thermal cyclers)

WARNING!

- For amplification use 384-well PCR microplates hermetically sealed with thermal film.
- The reagents and tubes must be kept away from direct sunlight.

8.2.1 **Package A.** Shake the tubes with PCR buffer Stream and TechnoTaq MAX polymerase on vortex for 3-5 seconds then centrifuge on vortex for 1-3 seconds. To run 48 tests, use 2 tubes with PCR buffer Stream and 2 tubes with TechnoTaq MAX polymerase. To run 24 tests, use 1 tube with PCR buffer Stream and 1 tube with TechnoTaq MAX polymerase.

Package A-TL. Shake the tubes with TL buffer on vortex for 3-5 seconds. Centrifuge the tubes with TL buffer and polymerase TL-65 on vortex for 1-3 seconds. To run 48 tests, use 2 tubes with TL buffer and 2 tubes with polymerase TL-65. To run 24 tests, use 1 tube with TL buffer and 1 tube with polymerase TL-65.

[4] - Please refer to Part II of the Operation manual for DTprime, DTprime II and DTlite real-time PCR instruments.

[5] - Instructions for downloading files with test parameters can be found on https://www.dna-technology.com/assaylibrary.

WARNING!

- Take TechnoTaq MAX polymerase out of the freezer immediately before use.
- For basic run, preparation of DNA samples and controls is required.

8.2.2 **For basic run**:

Package A. Prepare TechnoTaq MAX polymerase solution and DNA samples in accordance with the DTstream Operation manual.

Package A-TL. Prepare polymerase TL-65 solution and DNA samples in accordance with DTstream Operation manual.

8.2.3 **For integrated run:**

Package A. No preparation of TechnoTaq MAX polymerase solution and DNA samples is required.

Package A-TL. No preparation of polymerase TL-65 solution and DNA samples is required.

8.2.4 Centrifuge the tubes with PCR mix Stream on vortex for 1-3 seconds.

8.2.5 **For basic run:**

Place the tubes with PCR mix Stream based on the color marking of the mix (first tube of the strip is marked with blue buffer solution), tubes with prepared TechnoTaq MAX or polymerase TL-65 solution and PCR microplate onto the DTstream worktable.

8.2.6 **For integrated run:**

Package A. Set the tubes with PCR mix Stream based on the color marking of the mix (first tube of the strip is marked with blue buffer solution), tubes with PCR buffer Stream, TechnoTaq MAX polymerase solution, NA dilution buffer and PCR microplate onto the DTstream worktable.

Package A-TL. Set the tubes with PCR mix Stream based on the color marking of the mix (first tube of the strip is marked with blue buffer solution), tubes with TL buffer, polymerase TL-65, NA dilution buffer and PCR microplate onto the DTstream worktable.

8.2.7 Shake the tube with positive control on vortex for 3-5 seconds then centrifuge on vortex for 1-3 seconds.

WARNING!

- For extracted DNA and negative controls, follow the DNA preparation guidelines outlined in the instruction for use for the NA extraction kit (if necessary).
- For **PREP-NA PLUS** and **PREP-GS PLUS** (only in case the precipitate with the extracted DNA was transferred to new tubes) NA extraction kits: shake the tubes with the DNA sample and negative control on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.
- For **PREP-MB MAX DNA Extraction Kit**, spin the tubes with the DNA sample and negative control on vortex for 1-3 seconds (do not shake!), then place the tubes into magnetic rack. If after extraction the precipitate with the extracted DNA was transferred to new tubes, shake the tubes with the DNA sample and negative control on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

8.2.8 Place the DNA samples, positive and negative controls onto the DTstream worktable.

8.2.9 Open the strips with PCR mix Stream by carefully removing the protective foil.

8.2.10 Dose the reagents as specified in the Operation manual for DTstream.

8.2.11 When the DTstream dispensing protocol is completed, carefully place the PCR microplate onto the base of DTpack plate sealing device. Do not shake the PCR microplate!

8.2.12 Seal the PCR microplate with thermal film in accordance with the DTpack Operation manual.

8.2.13 Spin the PCR plate at RCF(g) 100 for 30 seconds.

8.2.14 Place the PCR microplate into the real-time PCR thermal cycler.

8.2.15 Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and IDs of the samples, positive and negative controls. Specify the position of the samples in the thermoblock and run PCR. Before the run, compare your selected program to the values provided in Table 9.

Table 9. DTprime and DTprime II amplification program (packages A, A-TL)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Step type
1	80	0	5	15		Cycle
	94	0	5			
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	25*	…	…	Temperature hold		Temperature hold
*temperature hold at 10°C is allowed						

[6] - Please refer to Part II of the Operation manual for DTprime, DTprime II and DTlite real-time PCR instruments.

[7] - Instructions for downloading files with test parameters can be found on https://www.dna-technology.com/assaylibrary.

9. CONTROLS

- **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** contains a positive control which is produced using genetic engineering techniques and contains the target DNA sequence.
- To reveal possible contamination, a negative control is required.

WARNING! The negative control must go through all stages of DNA extraction.

- The PCR mix contains control indicator (tube 5, Cy5 detection channel) which is based on the amount of human genomic DNA (HGD) present in the tube.
- The PCR mix also contains Total Bacterial Load (TBL) in tube 1, Cy5 channel, which quantifies the total amount of microorganisms present in the tube.
- The PCR mix from the kit contains an internal control (IC) in tubes 2-4, 6-8, Hex detection channel. IC is an artificial plasmid used to assess the quality of PCR performance.

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

The test result is considered valid if:

For positive control the test result must be displayed as a decimal logarithm of DNA concentration or as “+”, meaning “detected”. In case of a negative result (“-”, “not detected”) for the positive control the results of the current run are considered invalid. In this case it is required to repeat amplification of all DNA samples.

For negative control the test result must be displayed as “-”, meaning “not detected” for specific amplification target and as “+”, or “detected” for internal control. Otherwise, the results of the current run are considered invalid; decontamination procedure is required.

10. DATA ANALYSIS

10.1. Amplification results are calculated automatically by the software that is provided with the thermal cycler.

10.2. After amplification, the software compares the position of the tubes specified by the operator with the actual position of the marker. Software warns the operator if there is a mismatch. If so, it is necessary to check the position of the strips in the thermoblock (tube 1 of the strip is marked with a blue buffer solution) and correct the tube IDs in the protocol.

10.3. When analyzing the results, the values of TBL, HGD and internal controls must be considered:

10.3.1. If negative results are obtained in the same tube simultaneously for IC and for target analytes on the corresponding detection channels the assay, the result for that sample is considered invalid (except for tubes without IC). An invalid result may be due to the presence of inhibitors in the DNA sample obtained from biological material; incorrect execution of the assay protocol, inadequate amplification temperature, etc. In this case, it is necessary to either repeat PCR with the same DNA sample, repeat DNA extraction and amplification for this sample, or re-sample the biological material from the examinee (performed sequentially until the error is eliminated).

10.3.2. TBL and HGD values are used to determine whether the amount of biomaterial is sufficient to estimate the relative quantity of normal microbiota representatives and opportunistic microorganisms. For pathogenic microorganisms the result is considered valid regardless of the TBL and HGD values (10.7).

10.4. Test results for biomaterial samples containing DNA of target analytes, with the exception of pathogenic microorganisms, will be negative for the respective analytes ("-") and positive for IC (except for tubes without IC) if target DNA concentrations are below the detection threshold.

10.5. Results of the run are valid if the following conditions for controls are observed:

10.5.1. For negative control, a negative result ("-") must be obtained for the target analytes on the corresponding detection channels and a positive result must be displayed for IC (except for tubes without IC) on the Hex detection channel.

Results below detection threshold for all analytes, **except for** *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis*, HSV1, HSV2, CMV, are displayed as negative ("-").

If positive result is obtained for the negative control for target analytes the results of the entire run are considered invalid. In this case, measures should be taken to identify and eliminate possible contamination.

10.5.2. A positive result (5.0±1.0 Lg GE/mL) must be obtained for the positive control. The result obtained for the IC is considered irrelevant in this case.

If a negative result is obtained for a positive control, the results of the entire run are considered invalid. In this case it is necessary to repeat the amplification procedure for all samples.

10.6. Interpretation of the result consists of the following steps:

- Check the values for TBL and HGD. Lg GE/mL values for TBL and HGD <3.0 for cervical swabs and values <3.5 for vaginal swabs should be interpreted as an insufficient amount of biomaterial for this assay;
- For normal microbiota representatives *Lactobacillus iners*, *Lactobacillus* non-*iners,* opportunistic microorganisms *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Candida* spp*., Candida albicans, Gardnerella vaginalis, Fannyhessea vaginae, Mobiluncus* spp*., Staphylococcus* spp*., Streptococcus* spp*., Enterobacteriaceae, Enterococcus* spp*., Haemophilus* spp. the result may be negative if DNA concentration for the corresponding analytes is lower than limit of detection, or positive in two cases: "Detected, lower than limit of quantification" in case DNA concentration is lower than limit of quantification, and as an Lg value GE/mL of sample in case DNA concentration is higher than limit of quantification;
- To assess the composition of the microbiota, the proportion of the bacterial analyte (BA) relative to the total number of bacteria (TBL) is calculated using the formula

  $BA = 〚10〛^{Lg(BA)} / 〚10〛^{Lg(TBL)} \times 100\%,$

  where Lg (BA) and Lg (TBL) are the Lg values obtained for the target bacterial analyte and TBL, respectively.

  Using this formula, the proportion of bacterial groups relative to TBL can also be calculated. This includes the proportions of normal microbiota, aerobes (facultative anaerobic microorganisms) associated with vaginitis/cervicitis and anaerobes (obligate anaerobic microorganisms) associated with bacterial vaginosis. As a result, the dominant microorganism/group of microorganisms can be identified.

- for pathogenic microorganisms the result is considered independently from TBL and HGD values. It may be negative or positive in two cases: "Detected" ("+") in case DNA concentration is lower than limit of quantification, and as an Lg value GE/mL of sample in case DNA concentration is higher than limit of quantification;

  In case of significant differences between the values of total microorganisms detected and the TBL indicator, it is possible to conclude that there are microorganisms in the biomaterial sample that are not detected by the reagent kit.

11. SPECIFICATIONS

a. Analytical specificity

For biomaterial samples containing DNA of the target analytes, the software of the detecting thermal cycler records positive results for specific amplification products in the corresponding tubes on the specified detection channels during amplification.

For biomaterial samples that do not contain DNA of the target analytes, the software of the detecting thermal cycler records negative results for specific amplification products in the corresponding tubes on the specified detection channels and positive amplification results for the internal control on the Hex detection channel (except for tubes without IC, see Table 1).

Analytical specificity of **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit** was evaluated against a panel of microorganisms detectable by this reagent kit and those potentially present in biomaterial sample. The panel included microorganisms in concentrations of at least $1.0x10^6$ copies/mL. Absence of nonspecific positive amplification results and nonspecific cross-reactions of each component in the reagent kit against another target has been confirmed.

Table 10. Microorganisms used to assess analytical specificity

Microorganisms		
Anaerococcus vaginalis	*Fusobacterium nucleatum*	*Mycoplasma genitalium*
Bacteroides fragilis	*Gardnerella vaginalis*	*Neisseria gonorrhoeae*
Bifidobacterium bifidum	*Haemophilus influenzae*	*Parvimonas micra*
Burkholderia cepacia	HSV1	*Peptoniphilus asaccharolyticus*
BVAB1	HSV2	*Peptostreptococcus anaerobius*
BVAB2	HPV 16	*Porphyromonas vaginalis*
BVAB3	HPV 18	*Prevotella copri*
Candida albicans	HPV 45	*Propionibacterium acnes*
Candida parapsilosis	Human herpesvirus 6	*Proteus mirabilis*
Chlamydia trachomatis	Human herpesvirus 8	*Pseudomonas aeruginosa*
Corynebacterium vaginale	*Lactobacillus crispatus*	*Sneathia vaginalis*
CMV	*Lactobacillus iners*	*Staphylococcus aureus*
Dialister invisus	*Lactobacillus jensenii*	*Streptococcus agalactiae*
Eggerthella lenta	*Lactobacillus mulieris*	*Treponema pallidum*
Escherichia coli	*Lactobacillus gasseri*	*Trichomonas vaginalis*
Enterobacter intestinihominis	*Leptotrichia massiliensis*	*Ureaplasma urealyticum*
Enterococcus faecalis	*Megasphaera cerevisiae*	*Ureaplasma parvum*
Epstein-Barr virus	*Mobiluncus curtisii*	
Fannyhessea vaginae	*Mycoplasma hominis*	

b. Analytical sensitivity (limit of detection)

The limit of detection (LoD) of the DNA targets detectable by this reagent kit was established by analyzing limiting dilutions of quantitatively characterized reference controls containing vectors with specific DNA sequences of the target analytes (controlled for the copy number of target genes). The LoD was also confirmed by the results obtained on dilutions of quantitatively characterized microorganism cultures. Analytical sensitivity for different DNA targets is provided in Table 11.

WARNING! The LoD depends on the elution volume of the DNA extraction kits (the larger the elution volume, the higher the LoD, which is due to a decrease in DNA concentration).

Table 11. Analytical sensitivity for different DNA targets.

DNA targets	Analytical sensitivity in GE/mL
Trichomonas vaginalis	$1.0x10^2$
Candida spp., *Candida albicans*	$5.0x10^2$
TBL, HGD, *Lactobacillus iners, Lactobacillus* non-*iners, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Gardnerella vaginalis, Fannyhessea vaginae, Mobiluncus* spp*., Staphylococcus spp., Streptococcus spp., Enterobacteriaceae, Enterococcus spp., Haemophilus spp., Neisseria gonorrhoeae, Mycoplasma genitalium,* HSV1, HSV2, CMV	$1.0x10^3$

c. Quantification range, limit

Reagent kit analytes quantification range is from $1.0x10^4$ to $1.0x10^9$ GE/mL. It was established by analyzing limiting dilutions of quantitatively characterized reference controls containing vectors with specific DNA sequences of the detectable analytes (controlled for the copy number of target genes). The quantification range was confirmed by the results obtained on dilutions of quantitatively characterized microorganism cultures.

The limit of quantification of this reagent kit is the lower limit of the linear range of the amplification, which corresponds to a value of $1.0x10^4$ for all target analytes for the kit.

The coefficient of variation for the limit of quantification is no more than 10%.

d. Diagnostic characteristics

Diagnostic sensitivity and diagnostic specificity values with 95% confidence interval are indicated in Table 12.

Table 12. Diagnostic characteristics of **FEMOFLOR®DeltaScreen REAL-TIME PCR Detection Kit**

Biomaterial	DNA	Number of samples		D. sensitivity, %	D. specificity, %
		Positive	Negative		
Epithelial swab/scrape from the mucous membrane of cervical canal, vagina	*Chlamydia trachomatis*	100	300	100% (97.2-100)	100% (99.1-100)
	Neisseria gonorrhoeae	100	300	100% (97.2-100)	100% (99.1-100)
	Mycoplasma genitalium	100	300	100% (97.2-100)	100% (99.1-100)
	Trichomonas vaginalis	100	300	100% (97.2-100)	100% (99.1-100)
	HSV1	100	200	100% (97.2-100)	100% (98.6-100)
	HSV2	100	200	100% (97.2-100)	100% (98.6-100)
	CMV	100	200	100% (97.2-100)	100% (98.6-100)

Diagnostic characteristics of the kit for quantitative DNA-based detection of normal microbiota representatives *Lactobacillus iners, Lactobacillus* non-*iners,* quantitative DNA-based detection of opportunistic microorganisms *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Candida* spp*., Candida albicans, Staphylococcus* spp*., Streptococcus* spp*., Streptococcus agalactiae, Enterobacteriaceae, Enterococcus* spp*., Haemophilus* spp*., Gardnerella vaginalis, Fannyhessea vaginae, Mobiluncus* spp*.,* are not established due to the fact that the listed analytes may be present in the reproductive tract of healthy women, thus the kit only detects them quantitatively. For quantitative methods, diagnostic characteristics are not applicable.

e. Measurement precision (repeatability and reproducibility)

The repeatability and reproducibility of measurements of this kit were evaluated by testing model samples. Model samples are prepared using vaginal and cervical biological material as a matrix and adding known quantities of microorganisms to it. Four concentrations were used: the limit of detection, limit of quantification and two values within the linear range of the amplification curve – $1.0x10^6$ and $1.0x10^9$. Each sample underwent all stages of the assay (DNA extraction, amplification and results detection).

The **repeatability** was evaluated at each of the four concentrations, in each of the packages (S, A, A-TL), using one instrument (DTprime 5M* for package S, DTprime 5X* for packages A, A-TL), by one operator, over a period of 5 days, with 2 runs per day and two repeats per run. The same reagent kit batch for each package was used for all runs.

The coefficient of variation for the repeatability of measurements for the "limit of detection" concentration was no more than 20%, for all three concentrations in the linear range – no more than 10% for all packages.

The **reproducibility** of measurements was evaluated at each of the four concentrations, in each of the packages (S, A, A-TL). For package S, two types of instruments (DTprime 5M*, DTlite 5S*) - two of each type, for packages A, A-TL - three instruments of one type (DTprime 5X*) were used. Two batches of reagent kits of each package were used for all runs. All runs were performed by 2 operators on 2 different days.

The coefficient of variation for reproducibility of measurements for the "limit of detection" concentration was no more than 20%, for all three concentrations in the linear range – no more than 10% for all packages.

12. TROUBLESHOOTING

Table 13. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat the entire test Dispose of the current batch
C-	+	Contamination	Dispose of the current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat the entire test Re-take sample

If you encounter any undescribed issues, contact our customer service department:

Phone: +7(495) 640-16-93

E-mail: hotline@dna-technology.ru

https://dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures are performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640-17-71

E-mail: info@dna-technology.com

https://www.dna-technology.com

14. KEY SYMBOLS

	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Non-sterile

Annex A

Shortened procedure for DNA extraction from epithelial swabs from the mucous membrane of cervical canal and vagina using PREP-NA PLUS reagent kit

WARNING!

- Before starting work:
  - heat the thermostat to 65 °C;
  - take the **PREP-NA PLUS** reagent kit out of the refrigerator and check that there is no precipitate in the lysis solution. If precipitate is observed, heat the vial with the lysis solution on a thermostat preheated to 65°C to dissolve the precipitate completely. Then stir the solution by turning the vial upside down 5-10 times (avoiding foaming). Before use, cool the solution to room temperature (18°C to 25°C). The precipitate can also be dissolved at room temperature (18°C to 25°C) for approximately 12 hours.
- Caps may open during heating! Use tubes with self-locking caps or programmable thermostats with a clamp lid.

Procedure:

1. Mark a 1.5 mL plastic tube as negative control (C-).

2. Enter 300 µL of lysis solution into each marked tube with 100 µL of biomaterial and into the "C-" tube. Do not touch the edges of the tube.

3. Add 100 µL of negative control into the "C-" tube.

4. Close the tubes tightly and shake on vortex for 3-5 seconds.

5. Heat the tubes on thermostat at 65 °C for 5 minutes.

6. Spin the tubes on vortex for 3-5 seconds.

7. Add 400 µL of precipitation buffer solution into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3-5 seconds.

8. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18°C to 25°C) for 10 minutes.

9. Using a separate tip for each tube, carefully remove as much supernatant as possible without disturbing the precipitate.

10. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

11. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18°C to 25°C) for 1 minute.

12. Using a separate tip for each tube, carefully remove as much supernatant as possible without disturbing the precipitate.

13. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

14. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18°C to 25°C) for 1 minute.

15. Using a separate tip for each tube, carefully remove as much supernatant as possible without disturbing the precipitate. It is acceptable to leave up to 20-30 µL of liquid above the precipitate.

16. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

17. Add 300 µL of dilution buffer to the precipitate, shake the tubes on vortex for 3-5 seconds and centrifuge on vortex for 3-5 seconds.

18. Heat the tubes on thermostat at 65 °C for 5 minutes. Shake the tubes on vortex for 3-5 seconds.

19. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds at room temperature (from 18°C to 25°C) to spin down the condensate.

The DNA sample can now be introduced into PCR mix.

Before using the DNA sample for PCR, thaw the DNA sample and the negative control at room temperature (from 18°C to 25°C) or in a refrigerator (from 2°C to 8°C), then shake the tubes with the DNA sample and the negative control on vortex for 3-5 seconds, then centrifuge on vortex for 1-3 seconds.

WARNING! The DNA sample can be thawed only once!