DNA-TECHNOLOGY

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For research use only

N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P122-S3/9ER
R1-P122-23/9ER
R1-P122-UA/9ER
R1-P122-VA/XER

Version: 688-2.2026.01.12

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1. INTENDED USE

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is intended for detection of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* and *Mycoplasma genitalium* DNA in human biological material (epithelial cell swabs from the urogenital tract, urine, prostate fluid, ejaculate) by real-time PCR.

Application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit.**

Potential users: qualified personnel trained in molecular research methods.

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

2. METHOD

Method: polymerase chain reaction (PCR) with detection of the results in real time; multiplex qualitative analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their elongation by Taq-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot start is provided. Hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin for package S or the use of Taq-polymerase blocked by antibodies for packages A, U. Polymerase chain reaction only starts when paraffin is melted or thermal dissociation of a complex of Taq-polymerase and antibodies at 94°C is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the amplification mixture. When a specific product is formed, the DNA probe is destroyed and the effect of the quencher on the fluorescent label stops, which leads to an increase in the fluorescence level recorded by special devices. The number of destroyed probes (and therefore the fluorescence level) increases in proportion to the number of specific amplicons produced. The fluorescence level is measured at each amplification cycle in real time.

The PCR mix includes the internal control (IC), which is intended to assess the quality of the polymerase chain reaction.

The DNA probe used to detect the *Trichomonas vaginalis* amplification product DNA includes the fluorescent dye Fam. The DNA probe used to detect *Mycoplasma genitalium* amplification product DNA includes the fluorescent dye Rox. The DNA probe used to detect the *Chlamydia trachomatis* amplification product DNA includes the fluorescent dye Cy5. The DNA probe used to detect the *Neisseria gonorrhoeae* amplification product DNA includes the fluorescent dye Cy5.5. The DNA probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex.

The use of several fluorescent dyes allows to reduce the amount of tubes and biomaterial for the analysis as it is possible to record the results of different amplification reactions simultaneously in one tube.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Trichomonas vaginalis	IC	*Mycoplasma genitalium*	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*

Automatic analysis is available on instruments manufactured by "DNA-Technology": DTlite, DTprime or DTprime II REAL-TIME thermal cyclers for **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit is also approved for use with CFX96 (Bio-Rad, USA) real-time thermal cyclers.

3. CONTENT

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2–4.

Table 2. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package S, strips for R1-P122-S3/9ER and package S, tubes for R1-P122-23/9ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	20 µL in each	tubes, 12 strips of 8 or 96 individual tubes
Taq polymerase solution	Colorless transparent liquid	500 µL in each	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps[2]	12 strips of 8		

Table 3. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package U for R1-P122-UA/9ER

Reagent	Description	Total volume	Amount
PCR mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR buffer	Colorless transparent liquid	600 µL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

[1] - marking as C+ is allowed

[2] - for detection kit packaged in strips R1-P122-S3/9ER

Table 4. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package A, tubes for R1-P122-VA/XER

Reagent	Description	Total volume	Amount
PCR mix Stream	Colorless or pink transparent liquid	1.2 mL in each	4 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	100 µL in each	2 tubes
Positive control[3]	Colorless transparent liquid	130 µL	1 tube

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

The kit in package S is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The kit in package U is designed for 96 tests given that there are at least 5 samples per run (3 test samples, negative control and positive control).

The kit in package A is designed for 384 tests (one run of 384 tests or two runs of 192 tests), including test samples, negative controls and positive controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following equipment, reagents and consumables are required:

Equipment, reagents and consumables	Package S		Package U, dosing		Package A
	strips	tubes	manual	automated	tubes
UV PCR cabinet	yes	yes	yes	yes	yes
Real-time detecting thermal cycler [1]	yes	yes	yes	yes[2]	yes[2]
Vortex mixer	yes	yes	yes	yes	yes
Vortex rotor for 0.2 mL strips	yes	no	no	no	no
Refrigerator or cooling chamber	yes	yes	yes	yes	yes
Freezing chamber	no	no	yes	yes	yes
Tube rack for 1.5 mL tubes	yes	yes	yes	yes	yes
Tube rack for 0.2 mL tubes	no	yes	yes[3]	no	no
Tube rack for 0.2 mL stripped tubes	yes	no	no	no	no
Single channel pipettes (dispensers covering 0.5–10 µL, 2.0–20 µL, 20–200 µL, 200–1,000 µL volume range)	yes	yes	yes	yes	no
RNase and DNase free filtered pipette tips (volume 10 µL, 20 µL, 200 µL, 1000 µL)	yes	yes	yes	yes	no
Pipette rack	yes	yes	yes	yes	no
RNase and DNase free 1.5 mL microfuge tubes with caps	no	no	yes	yes	yes
RNase and DNase free 0.2 mL PCR tubes with caps or 96-well PCR microplate[4]	no	no	yes	no	no
Powder-free surgical gloves	yes	yes	yes	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes	yes	yes	yes
DTstream dosing instrument, version 12M1 or 15M1	no	no	no	yes	no
DTstream dosing instrument, version *M4	no	no	no	no	yes

[3] - marking as C+ is allowed

Equipment, reagents and consumables	Package S		Package U, dosing		Package A
	strips	tubes	manual	automated	tubes
RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream	no	no	no	yes	yes
DTpack plate sealing device	no	no	yes[5]	yes	yes
Centrifuge for microplates (RCF(g) at least 100)	no	no	yes[5]	yes	yes
Polymer thermal film for microplate sealing	no	no	yes[5]	yes	yes
384-well PCR microplate	no	no	no	yes	yes
Transport medium (if necessary), **STOR-F** (DNA-Technology, Russia) is recommended					
Physiological saline solution 0.9% NaCl (sterile) (if necessary)					
NA extraction reagent kits, the following are recommended: - **PREP-RAPID**, (not applicable to male urethral swabs); - **PREP-GS**; - **PREP-GS PLUS**; - **PREP-NA**; - **PREP-NA PLUS**; - **PREP-OPTIMA**; - **PREP-MB MAX**; - **PREP-MB-RAPID II**.					
Notes: [1] – hereinafter – detecting thermal cycler; the required parameters are indicated below [2] – DTprime 5X* and DTprime II 5X* (manufactured by "DNA-Technology R&P", LLC) are validated [3] – only if using tubes [4] – not used with DTlite detecting thermal cycler [5] – if using PCR micoplates					

The following detecting thermal cyclers are validated for work with the **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex 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), (only for package U, automated dosing, and package A) hereinafter – DTprime 5X*;
- DTprime II in DTprime II 5X* modification (manufactured by "DNA-Technology R&P", LLC), (only for package U, automated dosing, and package A) hereinafter – DTprime II 5X*;
- DTlite in DTlite 5S* modification (manufactured by "DNA-Technology R&P", LLC), (only for package S and package U, manual dosing), hereinafter – DTlite;
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), (only for package S and package U), hereinafter – CFX96.

For the use of detecting thermal cyclers other than those listed above, please consult the reagent kit manufacturer for consultation.

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. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Storage conditions

5.1.1. Package S

– All components of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex 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 out of light over the storage period.

5.1.2. Packages U, A, tubes

- All components of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex 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 must be stored out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at the temperatures from minus 22°C to minus 18°C 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. Packages U, A, tubes

– 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.

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. Packages U, A, tubes

- All components of the kit, except 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 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 at temperatures from minus 22°C to minus 18°C over the storage period.

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

An expired **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** must not be used.

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

The conformity of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

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 test. 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[4, 5]. 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.

[4] - 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.

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

- 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 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

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from epithelial cell swabs from the urogenital tract, urine, prostate fluid, ejaculate.

General requirements

- PCR analysis is a direct method of laboratory analysis, thus human biomaterial sample collection must be performed from the lesion of infection.
- The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
- If biomaterial must be taken from several biotopes, repeat the procedure taking material into a new tube each time.
- Incorrect biomaterial sampling may lead to unreliable results and, thus, to the necessity of repeated sampling.
- During biomaterial preparation use RNase and DNase free filter pipette tips.

- To prevent contamination, only open the cap of the tube where biomaterial is introduced (adding sample/reagent, supernatant removal) and close it before proceeding to the next tube.

Interfering substances

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

PCR inhibitors are: hemoglobin, medications present in the DNA sample as a result of incomplete removal during the extraction of DNA from the biomaterial sample, isopropyl alcohol and methyl acetate present in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control sample and internal control are: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL of the DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

To assess the possible interference of drugs, we selected those potentially present in residual amounts in human biological samples taken from the corresponding biotopes under analysis (Miramistin®, chlorhexidine bigluconate).

For all the drugs under analysis no effect was found in concentration up to 10% in biomaterial sample.

Sample collection

Epithelial cell swabs from urogenital tract (cervical canal, vagina, urethra)

Urogenital swabs

Women should not carry out genitals' toilet and vaginal douching the day before research. To obtain an objective result, it is necessary that the material contains the largest count of epithelial cells and the minimum amount of mucus and blood impurities. Incorrect intake of biological material can lead to uncertain results and, therefore, to re-sample of biomaterial.

WARNING! Before obtaining a scrape of epithelial cells from urethra, posterior vaginal fornix, and cervical canal, free-flowing secretion should be removed with a sterile cotton swab.

Posterior vaginal vault

The material should be taken before physical inspection. Speculum can be moistened with hot water before manipulation, the use of antiseptics for speculum treatment is contraindicated. Scraping is taken from posterior vaginal vault. In case of virginal women, scrape is taken from vestibular mucous membrane and in some cases from posterior vaginal vault through hymenal rings.

Urethral sampling

Before sampling procedure, the examinee is recommended to refrain from urination for 1.5–2 hours.

Immediately before sampling procedure, it is necessary to treat external urethral orifice with a tampon moistened with sterile physiological solution.

In the presence of purulent discharge, sample must be taken 15–20 minutes after urination. In the absence of discharge, it is necessary to massage the urethra with sampling swab or brush. In case of women, the swab or brush is inserted to a depth of 1.0–1.5 cm, in case of children, the material is taken only from the external urethral orifice.

Cervical sampling

Before sampling procedure, it is necessary to remove mucus with a cotton tampon and, then, treat cervix with sterile physiological solution. The sampling swab is inserted into cervical canal to a depth of 0.5–1.5 cm. Removing the swab, contact of the walls of vagina should be excluded.

Urogenital swabs (cervical canal, vagina, urethra)

Procedural limitations — local application of medicines, vaginal ultrasound less than 24 hours before the procedure.

Sampling procedure is carried out using special sterile disposable instruments – urogenital swabs, cytobrushes or tampons, depending on the source of biological material in accordance with established procedures.

WARNING! In case of pregnancy the use of cytobrushes is contraindicated.

The taking of swabs is carried out:

- in plastic 1.5 mL tubes with 300–500 µL of sterile physiological solution;
- in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR;
- in tubes with **PREP-RAPID**.

N o t e . **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

WARNING! Take material into tubes with **PREP-RAPID** reagent using a dry swab! Solutions must not contact with skin, eyes and mucous membranes.

Order of taking:

1. Open the tube.
2. Move the swab with biological material to the tube with physiological solution, transport medium,
   or **PREP-RAPID**, and rinse it thoroughly, avoiding splashing of liquid. Then, remove the swab from the solution, pressing it to the wall of the tube, press out the excess liquid, remove the swab and discard. In the case of taking biomaterial from several biotopes, repeat the procedure, taking the material with a new swab into a new tube each time.
3. Close the tube tightly, mark the tube.

First portion of morning urine

First portion of morning urine as a biological material is used in acute inflammation of the lower urinary tract due to pain of taking swabs of epithelial cells.

First portion of morning urine in the amount of 10–15 mL is selected for the analysis. It is possible to examine first portion of urine received 2 or more hours after the previous urination.

Urine is taken into a special dry sterile container with a volume of up to 60 mL, equipped with a hermetical screw-cap.

After urine collection, container is tightly screwed and marked.

Prostate fluid

Before taking prostate fluid, sexual abstinence is recommended for 3 days before the procedure.

Before taking prostate fluid, penis balanus is treated with a sterile cotton tampon moistened with a physiological solution.

Prostate fluid is collected after prostate massage through rectum. Massage is performed by a specialist, by means of vigorous pressing movement from the base to the top of the gland.

After the end of massage, released prostate fluid in the form of a free-flowing drop (0.15–1.0 mL) is collected in a 2.0 mL single dry sterile tube or a container with a volume of up to 60 mL.

The container with prostate fluid is hermetically screwed and marked.

WARNING! Suspecting acute prostatitis, the prostate massage is strictly prohibited!

Ejaculate

Before collecting ejaculate (seminal fluid), sexual abstinence is recommended for 3 days before the examination.

Before collecting ejaculate, examinee urinates in the toilet, completely emptying the bladder.

After urinating, examinee should wash his hands thoroughly with soap and wash external genitals with soap and water. Penis balanus and foreskin should be dried with a sterile napkin.

Ejaculate is obtained by masturbation and collected in a sterile container with a volume of up to 60 mL.

The container with ejaculate is hermetically closed and marked.

Transportation and storage of samples

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

In case of usage of transport media, biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

WARNING! Only one freezing-thawing of material is allowed.

Sample preparation

Taking swabs in tubes with **PREP-RAPID**, pretreatment is not required. The material is ready for DNA extraction.

Biomaterial pretreatment is performed in accordance with **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS, PREP-RAPID, PREP-OPTIMA, PREP-MB MAX** and **PREP-MB-RAPID II** extraction kits instructions for use.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-RAPID, PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS**, **PREP-OPTIMA, PREP-MB MAX** and **PREP-MB-RAPID II** extraction kits are recommended. See Table 5.

Table 5. Reagent kits recommended for DNA extraction for further analysis with **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex Detection Kit**

Reagent kit	Set	Minimum eluate volume, µL
PREP-NA/PREP-NA PLUS	PREP-NA, PREP-NA (shortened method in accordance with Annex A)	50
	PREP-NA PLUS, PREP-NA PLUS (shortened method in accordance with Annex A)	300
PREP-GS/PREP-GS PLUS	PREP-GS	100
	PREP-GS PLUS	300
PREP-RAPID	PREP-RAPID	500
PREP-OPTIMA	PREP-OPTIMA, PREP-OPTIMA (shortened method in accordance with Annex B)	400
PREP-MB MAX	PREP-MB MAX	50–300
PREP-MB-RAPID II	PREP-MB-RAPID II	100

DNA extraction from the biomaterial is performed in accordance with the instruction for use to the NA extraction kit or with Annexes A, B in case of using **PREP-NA/PREP-NA PLUS** and **PREP-OPTIMA** kits for shortened methods of DNA extraction.

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

Analysis procedure

8.1. PCR, package S

WARNING! Reagents and tubes should be kept away from direct sunlight.

WARNING! When using package S (R1-P122-S3/9ER), strips, strictly observe the completeness of strips and caps for them. Do not use caps to the strips from other kits!

8.1.1. Mark tubes/strip tubes with paraffin-sealed PCR mix for each test sample, negative control ("C-") and positive control ("C+").

WARNING! The volume of reagents in the kit is intended for no more than 24 runs considering a variable number of test samples, 1 negative control and 1 positive control for each run.

Example: to test 4 samples, mark 4 tubes/strip tubes for samples, 1 tube for "C-" and 1 tube for "C+". The resulting number of tubes/strip tubes is 6.

8.1.2. Shake the tube with Taq polymerase solution for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

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

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

8.1.5. Shake the tube with positive control for 3–5 seconds on vortex mixer and centrifuge for 1–3 seconds on vortex.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes with reaction mixture, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA PLUS**, **PREP-GS** and **PREP-GS PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA preparation and negative control on vortex mixer for 3–5 seconds and centrifuge for 1–3 seconds on vortex.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1–3 seconds and place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.
4. To prevent contamination, before adding DNA only open the caps of the tubes where the sample is going to be introduced, and close the tube before proceeding to the next one. In case of using tubes in strips, close the strip after introducing samples before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.1.6. Add 5.0 µL of DNA preparation, extracted from DNA, into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.1.7. Add 5.0 µL of negative control ("C-") which passed whole DNA extraction procedure into corresponding tube. Avoid paraffin layer break.

8.1.8. Add 5.0 µL of positive control ("C+") into corresponding tube. Avoid paraffin layer break.

8.1.9. Centrifuge tubes/strips on vortex for 1–3 seconds.

8.1.10. Set the tubes/strips into the real-time thermal cycler.

8.1.11. **For DT thermal cycler**:

Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and IDs of the samples, negative and positive controls. Specify the position of the tubes/strips in the thermal unit (8.1.10) and run PCR. See Table 6.

For CFX96 (Bio-Rad) thermal cycler:

Perform PCR with 35 µL of reaction mixture. See Table 7.

Table 6. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (package S)

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	25[1]			Holding		Holding
√ — optical measurements [1] — holding at 10°C is allowed						

Table 7. Amplification program for CFX96 thermal cycles (package S)

№ of block (Step)	Temperature, °C	Time min:sec	Number or cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ — optical measurements (Plate Read), set measurement of fluorescence on Fam, Hex, Rox, Cy5 and Cy5.5 channels at 64°C			

8.2. PCR, package U, manual dosing

WARNING!

1. For amplification, use 0.2 mL amplification tubes or 96-well sealed PCR microplates[8]. The use of strips is not recommended due to post-amplification contamination.
2. Reagents and tubes should be kept away from direct sunlight.

[6] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite real-time PCR instruments PART II.
[7] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.
[8] - 96-well PCR microplates are not used with DTlite detecting thermal cyclers

8.2.1 Mark the required number of 0.2 mL amplification tubes or a 96-well PCR microplate for test samples, negative control ("C-") and positive control ("C+").

N o t e . It is recommended to test at least 5 samples per test (3 test samples, negative and positive controls).

Example: to test 4 test samples, mark 4 tubes/microplate wells for samples, 1 tube/well for "C-" and 1 tube/well for "C+". The resulting number of tubes/wells is 6.

8.2.2 Shake the tube with PCR mix for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

8.2.3 Add 6.0 µL of PCR mix to each tube/well, including "C-" and "C+".

8.2.4 Shake the tubes with PCR buffer and TechnoTaq MAX polymerase for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

WARNING! Take TechnoTaq MAX polymerase out from the freezing chamber immediately prior to use.

8.2.5 Prepare a mixture of PCR buffer and TechnoTaq MAX polymerase. Add into one tube:

6.0 x (N+1) µL of PCR buffer,

0.3 x (N+1) µL of TechnoTaq MAX polymerase,

where N is the quantity of marked tubes/microplate wells considering "C-", "C+".

Example: for simultaneous testing of 4 test samples, "C-" and "C+" in one PCR run, mark 6 tubes/microplate wells.

Prepare a mixture of PCR buffer and TechnoTaq MAX polymerase for 7 (6+1) tubes/wells. Mix 42 µL of PCR buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Shake the tube with the mixture of PCR buffer and TechnoTaq MAX polymerase for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

WARNING! Mixture of PCR buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR buffer and TechnoTaq MAX polymerase mixture into each marked tube/well with PCR mix. Close the tubes.

WARNING! Follow the steps listed in pp. 8.2.8–8.2.14 within two hours after adding PCR buffer and TechnoTaq MAX polymerase mixture to the tubes/wells with PCR mix.

8.2.8 Shake the tube with positive control for 3–5 seconds on vortex mixer and centrifuge for 1–3 seconds on vortex.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes/wells with PCR mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA PLUS**, **PREP-GS** and **PREP-GS PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA- preparation and negative control on vortex mixer for 3–5 seconds and centrifuge for 1–3 seconds on vortex.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1–3 seconds and place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.
4. To prevent contamination, before adding DNA only open the caps of the tubes where the sample is going to be introduced, and close the tube before proceeding to the next one. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA preparation samples into marked tubes/microplate wells. Do not add DNA into the "C-", "C+" tubes.

8.2.10 Add 6.0 µL of negative control (“C-”) which passed whole DNA extraction procedure into the corresponding tube/well.

8.2.11 Add 6.0 µL of positive control (“C+”) into the corresponding tube/well.

8.2.12 **In case of using a 96-well PCR microplate**:

8.2.12.1. Place the microplate into the plate carrier of DTpack plate sealing device.

8.2.12.2. Seal PCR microplate with polymer thermal film in accordance with DTpack operation manual.

8.2.12.3. Spin PCR microplate at RCF(g) 100 for 30 seconds.

8.2.13 **In case of using tubes**:

Centrifuge the tubes for 3–5 seconds on vortex mixer.

8.2.14 Set the tubes into real-time thermal cycler and run PCR.

8.2.15 **For DT thermal cycler**:

Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and IDs of the samples, negative and positive controls. Specify position of the tubes/plate in thermal unit and run PCR. The volume of reaction mixture is 18 µL. See Table 8.

For CFX96 (Bio-Rad) thermal cycler:

Perform PCR with 18 µL of reaction mixture. See Table 9.

Table 8. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (packages U, A)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
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[1]			Holding		Holding
√ — optical measurements [1] — holding at 10°C is allowed						

Table 9. The PCR program for CFX96 thermal cyclers (package U)

№ of block (Step)	Temperature, °C	Time min:sec	Number or cycles (repeats)
1	80	01:00	1
2	94	05:00	1
3	94	0:15	50
4	64 √	0:20	
√ — optical measurements (Plate Read), set measurement of fluorescence on Fam, Hex, Rox, Cy5 and Cy5.5 channels at 64°C			

[9] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite real-time PCR instruments PART II.
[10] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.3. PCR, package U, using DTstream 12M1 or 15M1 (only for DTprime 5X* and DTprime II 5X* thermal cyclers)

WARNING!

1. For amplification use 384-well PCR microplates hermetically sealed with thermal film.
2. Reagents and tubes should be kept away from direct sunlight.

N o t e — It is recommended to test at least 5 samples per test (3 test samples, negative and positive controls).

8.3.1 Shake the tube with PCR mix for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

8.3.2 Shake the tube with PCR buffer and TechnoTaq MAX polymerase for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

WARNING! TechnoTaq MAX polymerase should be taken out from the freezing chamber immediately prior to use.

8.3.3 Prepare the mixture of PCR buffer and TechnoTaq MAX polymerase in a separate tube according to the user manual for dosing device DTstream.

8.3.4 Shake the tube with the mixture of PCR buffer and TechnoTaq MAX polymerase for 3–5 seconds on vortex mixer, then centrifuge for 1–3 seconds on vortex.

8.3.5 Shake the tube with positive control (“C+”) for 3–5 seconds on vortex mixer and centrifuge for 1–3 seconds on vortex.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes with PCR mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA PLUS**, **PREP-GS** and **PREP-GS PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA-preparation and negative control on vortex mixer for 3–5 seconds and centrifuge on vortex for 1–3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1–3 seconds and place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control “C-” on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

8.3.6 Set the tubes with PCR mix, the mixture of PCR buffer and TechnoTaq MAX polymerase, tubes or deep-well plate with DNA sample preparations, positive and negative controls and PCR microplate onto the DTstream working table and perform dosing of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the plate carrier of DTpack sealing instrument.

8.3.8 Run sealing of PCR microplate according to DTpack user manual.

8.3.9 Spin the microplate on RCF(g) 100 for 30 seconds.

8.3.10 Set the PCR microplate into real-time thermal cycler.

8.3.11 Launch the operating software for DT instrument[11]. Add corresponding test[12], specify the number and IDs of the samples, negative and positive controls. Specify position of the PCR microplate in thermal unit and run PCR. See Table 8.

8.4. PCR, package A, using DTstream *M4 (only for DTprime 5X* and DTprime II 5X* thermal cyclers)

WARNING!

1. For amplification, use 384-well sealed PCR microplates.

2. Reagents and tubes should be kept away from direct sunlight!

8.4.1 Shake the tubes with PCR mix Stream and TechnoTaq MAX polymerase on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds. For 384 tests, use 4 tubes with PCR mix Stream and 2 tubes with TechnoTaq MAX polymerase. For 192 tests, use 2 tubes with PCR mix Stream and 1 tube with TechnoTaq MAX polymerase.

WARNING! TechnoTaq MAX polymerase should be taken out from the freezing chamber immediately prior to use.

8.4.2 Shake the tube with positive control (“C+”) for 3–5 seconds on vortex mixer and centrifuge for 1–3 seconds.

WARNING! Before introducing DNA preparation and negative control into the tubes with PCR mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.

8.4.3 Install deep-well plates with DNA samples and negative controls onto the DTstream working table according to the run protocol.

8.4.4 Set the tubes with PCR mix Stream, TechnoTaq MAX polymerase and positive control, as well as PCR microplate onto the DTstream working table and perform dosing of components according to DTstream user manual.

8.4.5 After the end of dosing program on DTstream put the PCR microplate without shaking on the plate carrier of DTpack sealing instrument.

8.4.6 Run sealing of PCR microplate according to DTpack user manual.

8.4.7 Spin the microplate on RCF(g) 100 for 30 seconds.

8.4.8 Set the PCR microplate into real-time thermal cycler.

8.4.9 Launch the operating software for DT instrument[13]. Add corresponding test[14], specify the number and IDs of the samples, negative and positive controls. Specify position of the PCR microplate in thermal unit and run PCR. See Table 8.

[11] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite real-time PCR instruments PART II.

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

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

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

9. CONTROLS

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** contains positive control. 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 quality of PCR performance.

To reveal possible contamination, a negative control is required.

WARNING! A negative control should go through all stages of DNA extraction. Physiological saline solution or negative control from an extraction kit can be used as a negative control in volumes indicated in supplied instructions.

For **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** the test result is considered valid when:

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

For **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** the test result is considered invalid when exponential growth of fluorescence level for specific product and for internal control is not observed.

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

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

10. DATA ANALYSIS

10.1. Registration of the results is carried out automatically during amplification by the software provided with detecting thermal cyclers.

10.2. When using CFX96 (Bio-Rad) detecting thermal cyclers, use regression type analysis (Cq Determination Mode: Regression); in the "Baseline Setting" tab choose "Baseline Subtracted Curve Fit".

10.3. Result interpretation is performed in accordance with Table 10. Run results are valid, if the result interpretation conditions for controls are observed.

Table 10. Interpretation of PCR results

Detection channel					**Result interpretation**
Fam, Cp/Cq	Hex, Cp/Cq	Rox, Cp/Cq	Cy5, Cp/Cq	Cy5.5, Cp/Cq	
Test samples					
Specified	Not considered	Not specified	Not specified	Not specified	***Trichomonas vaginalis* DNA is detected**
Not specified	Not considered	**Specified**	Not specified	Not specified	***Mycoplasma genitalium* DNA is detected**
Not specified	Not considered	Not specified	**Specified**	Not specified	***Chlamydia trachomatis* DNA is detected**
Not specified	Not considered	Not specified	Not specified	**Specified**	***Neisseria gonorrhoeae* DNA is detected**
Not specified	**Specified**	Not specified	Not specified	Not specified	DNA of the sought microorganisms is not detected
Not specified	Not specified	Not specified	Not specified	Not specified	Unreliable result
Negative control					
Not specified	**Specified**	Not specified	Not specified	Not specified	**Negative result** Run results are valid
Positive control					
Specified	Not considered	**Specified**	**Specified**	**Specified**	**Positive result** Run results are valid

10.4. Unreliable result may be due to inhibitors in DNA preparation obtained from biological material; incorrect analysis protocol performance; non-compliance of amplification temperatures etc. In this case, either a repeated PCR with the available DNA preparation is required, or a repeated biological material sampling (performed sequentially).

10.5. If Cp/Cq values obtained for biomaterial sample are less than 24 on Fam, Rox, Cy5 or Cy5.5 detection channels, this indicates a high initial DNA concentration of the corresponding microorganism. In this case, it is possible to obtain a false negative result for a microorganism whose DNA is present at a low concentration. To exclude false negative results, it is recommended to repeat PCR of the extracted DNA preparation using the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit**, **Trichomonas vaginalis REAL-TIME PCR Detection Kit**, **Chlamydia trachomatis REAL-TIME PCR Detection Kit** and **Mycoplasma genitalium REAL-TIME PCR Detection Kit**.

10.6. If a positive result is obtained for negative control, results of the whole run are considered invalid. In this case special measures for identification and elimination of a possible contamination are necessary.

10.7. If a negative result is obtained for positive control, results of the whole run are considered invalid. In this case repeated amplification for all samples is required.

11. SPECIFICATIONS

a. Analytical specificity

In human biomaterial samples containing the DNA of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* or *Mycoplasma genitalium*, the detection thermal cycler software registers positive amplification results for the specific product on the corresponding detection channel.

In human biomaterial samples not containing the DNA of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* or *Mycoplasma genitalium*, the detection thermal cycler software registers negative amplification results for the specific product and positive result for the internal control (IC).

The absence of nonspecific positive amplification results has been shown in high DNA concentrations of closely related microorganisms or microorganisms potentially present in the test samples: *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum, Candida albicans, Streptococcus* spp., *Staphylococcus* spp., and human DNA in concentration up to 1.0×10^8 copies/mL of the sample.

There was no viable inhibition when studying samples containing non-specific DNA in concentration of up to 1.0×10^8 copies/mL of the sample and *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* and *Mycoplasma genitalium* DNA in concentrations of up to 1.0×10^3 copies/mL of the sample.

b. Limit of detection (LOD)

LOD is 5 copies of each microorganism DNA per amplification tube.

LOD is determined by analysis of serial dilutions of two laboratory controls (LC).

LOD for test sample depends on the used DNA extraction kit and the volume of obtained DNA preparation:

Biomaterial	DNA extraction reagent kit	Obtained preparation volume, µL	Limit of detection, copies/sample
Epithelial cell scrapes in 500 µL of transport medium[15]	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
	PREP-GS	100	100
	PREP-GS PLUS	300	300
	PREP-RAPID	500	500
	PREP-OPTIMA	400	400
	PREP-MB MAX	50–300	100
	PREP-MB-RAPID II	100	100
Urine (extraction from 1.0 mL of sample)	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
	PREP-GS	100	100
	PREP-GS PLUS	300	300
	PREP-RAPID	500	500
	PREP-OPTIMA	400	400
	PREP-MB MAX	50–300	100
	PREP-MB-RAPID II	100	100
Ejaculate (extraction from 100 µL of sample)	**PREP-NA**	50	50
	PREP-NA PLUS	300	300
	PREP-GS	100	100
	PREP-GS PLUS	300	300

[15] - STOR-F (DNA-Technology, Russia) was used

Biomaterial	DNA extraction reagent kit	Obtained preparation volume, µL	Limit of detection, copies/sample
Prostate fluid (extraction from 100 µL of sample)	**PREP-OPTIMA**	400	400
	PREP-MB MAX	50–300	100
	PREP-NA	50	50
	PREP-NA PLUS	300	300
	PREP-GS	100	100
	PREP-GS PLUS	300	300
	PREP-RAPID	500	500
	PREP-MB MAX	50–300	100

c. Diagnostic characteristics

Analyte	Diagnostic characteristics	
	Diagnostic sensitivity (%)	**Diagnostic specificity (%)**
Trichomonas vaginalis	96.30% (95CI 81.03 – 99.91)	98.26% (95CI 93.86 – 99.79)
Mycoplasma genitalium	100% (95CI 86.26 – 100)	100% (95CI 96.90 – 100)
Chlamydia trachomatis	100% (95CI 89.11 – 100)	99.09% 95CI (95.04 – 99.98)
Neisseria gonorrhoeae	96.00% (95CI 79.65 – 99.90)	100% (95CI 96.90 – 100)

d. Repeatability

The general repeatability of results obtained using the reagent kit was 100%.

NOTE. The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS**, **PREP-RAPID**, **PREP-OPTIMA, PREP-MB MAX** and **PREP-MB-RAPID II** extraction kits.

12. TROUBLESHOOTING

Table 11. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose of the current batch
C-	+	Contamination	Dispose of the 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:

E-mail: hotline@dna-technology.ru
https://www.dna-technology.com/support

13. QUALITY CONTROL

The quality controlprocedures performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Contact our customer service with quality issues of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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14. KEY TO 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 method of NA extraction from the test biomaterial (epithelial cell swabs from urogenital tract) using PREP-NA, PREP-NA PLUS extraction kits

WARNING!

1. Before starting work it is necessary to:
   - preheat the thermostat to 65°C;
   - take out of the refrigerator the NA extraction reagent kit and check the absence of precipitate in the lysing solution. In case of precipitation it is necessary to heat the vial with lysis solution on the thermostat preheated to 65°C, until complete dissolution of the precipitate. Then stir the solution by turning the vial upside down 5–10 times, avoid 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) within approximately 12 hours.
2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

Procedure:

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

2. Add 300 µL of lysis solution into each marked tube with 100 µL of pretreated samples and into the "C-" tube. Avoid touching the walls of the tubes.

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 into each tube, 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. Remove supernatant as fully as possible using separate tip for each tube. Avoid touching the precipitate.

10. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and stir by turning tubes gently 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. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.

13. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and stir by turning tubes gently 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. Remove supernatant using separate tip for each tube. Avoid touching the precipitate. It is allowed to leave up to 20–30 µL of liquid covering the precipitate.

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

17. Add 50 µL (**PREP-NA**) or 300 µL (**PREP-NA PLUS**) of dilution buffer, 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 at room temperature (from 18°C to 25°C) for 30 seconds to spin down the condensate.

DNA preparation is ready to be introduced into the PCR mix.

DNA preparation can be stored at temperature from minus 22°C to minus 18°C for up to 1 month or at temperature from minus 72°C to minus 68°C for up to 1 year.

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

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready to be introduced into the PCR mix.

Annex B

Shortened method of NA extraction from the test biomaterial (epithelial cell swabs from urogenital tract) using PREP-OPTIMA extraction kit

WARNING!

1. Switch on the thermostat and preheat it to 90°C.
2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

Note - For thermostat a program with active final cooling is recommended; otherwise, take the tubes carefully out of the thermostat and let them cool down in an upright position to room temperature (from 18°C to 25°C).

1. In case of biomaterial intake into the STOR-F transport medium:

1.1. Centrifuge the tubes with test samples in transport medium and negative control at RCF(g) 12,000–16,000 for 1 minute.

1.2. Remove transport medium as fully as possible using a separate tip for each tube leaving up to 50 µL of precipitate + liquid fraction in the tubes. Avoid touching the precipitate.

1.3. Add 400 µL of lysis solution into each tube.

1.4. Proceed to points 2.1–2.7.

2. In case of biomaterial intake into lysis solution:

2.1. Mix the content of tubes with test samples and negative control thoroughly on vortex for 10–30 seconds.

2.2. Spin down the drops from tube caps on vortex for 10–30 seconds.

2.3. Heat the tubes on thermostat to 90°C for 5 minutes.

2.4. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 minute.

2.5. Shake the tubes with neutralizing solution on vortex for 1–3 seconds. Centrifuge on vortex for 1–3 seconds.

2.6. Add 8.0 µL of neutralizing solution into each tube (2.0 µL of neutralizing solution per 100 µL of sample). Avoid touching the walls of the tubes.

2.7. Mix the content of tubes thoroughly on vortex for 10–30 seconds and centrifuge on vortex for 1–3 seconds.

DNA preparation is ready to be introduced into the PCR mix.

DNA preparation can be stored at temperature from 2°C to 8°C for up to 1 month or at temperature below minus 18°C for up to 6 months.

Before using DNA preparation for PCR after storage, thaw DNA preparation and negative control at room temperature (from 18°C to 25°C) or at temperature from 2°C to 8°C, then shake the tubes with DNA preparation and negative control on vortex for 1–3 seconds and centrifuge at RCF(g) 12,000–16,000 for 1 minute.

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready to be introduced into the PCR mix.