DNA-TECHNOLOGY

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For research use only

T.vaginalis/M.genitalium

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF R1-P126-S3/9EU
R1-P126-23/9EU
R1-P126-UA/9EU

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1. INTENDED USE

The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is intended for simultaneous detection of *Trichomonas vaginalis* and *Mycoplasma genitalium* DNA in human biological material (scrapes of epithelial cells from the urogenital tract) by real-time PCR.

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

The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this 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 extension 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 package U. Polymerase chain reaction only starts when paraffin is melted or thermal dissociation of a complex of Taq polymerase and antibodies 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 probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex. 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*	-	-

Automatic analysis is available on instruments manufactured by "DNA-Technology": DTlite or DTprime REAL-TIME thermal cyclers for **T.vaginalis/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.

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

3. CONTENT

The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control. Detailed description of content is represented in Tables 2, 3.

Table 2. The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package S, strips for R1-P126-S3/9EU and package S, tubes for R1-P126-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1,920 µL (20 µL in each)	tubes, 12 strips of 8 or 96 individual tubes
Taq-polymerase solution	Colorless transparent liquid	1,000 µL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (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 **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package U for R1-P126-UA/9EU

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

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

The kit in package S is intended for single use and designed for 96 tests (including one negative control and one positive control in each run). It is recommended to perform no more than 24 runs.

The kit in the package U is intended for 96 tests and requires at least 5 samples in a single run (3 test samples, negative control and positive control).

[1] - marking as C+ is allowed

[2] - for detection kit packaged in strips REF R1-P126-S3/9EU

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, sterile single use flasks and sterile containers to collect test material;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) at least 12,000);
- Solid-state thermostat (temperature range 50-98 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-NA-PLUS** (REF P-002/2EU), **PREP-GS**(REF P-003/1EU), **PREP-GS-PLUS** (REF P-003/2EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs), **PREP-MB-RAPID** (REF P-116-N/4EU, P-116-A/8EU), **PREP-OPTIMA** (REF P-016-1/2EU, REF P-016-N/2EU) and **PREP-MB-RAPID II** (REF P-122-A/9EU, P-122-N/9EU, P-122-P/9EU, P-124-P/9EU) extraction kits are recommended;
- Physiological saline solution 0.9% NaCl (sterile) (if needed);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL ,200 µL, 1000 µL);
- Pipette stand;
- Magnetic homogenizer (in case of using **PREP-MB-RAPID** and **PREP-MB-RAPID II** extraction kit);
- System for automatic nucleic acid extraction in 96 deep-well plate (Allsheng Auto-Pure 96 or KingFisher Flex) (in case of using **PREP-MB-RAPID II** extraction kit);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber (in case of using package U);
- Vortex mixer;

- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- 0.2 mL PCR tubes (in case of using package U);
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (dispensers covering 0.5-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- DTstream *M1 dosing device ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- 96-well microplates (only for manual dosing in case of using package U; are not used with DTlite detecting thermal cycler);
- 384-well microplate (only for automated dosing in case of using package U);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-amplification – amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to storage conditions of the kit components.

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

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

All components of **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit**, except TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C over the storage period. PCR-mix and paraffin sealed PCR-mix must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C and out of light over the storage period. TechnoTaq MAX polymerase must be stored at the temperature from minus 22 °C to minus 18 °C over the storage period.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit, except TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C over the storage period;
- TechnoTaq MAX polymerase must be stored at temperatures from minus 22 °C to minus 18 °C over the storage period;
- PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light over the storage period.

The kit stored under undue regime must not be used.

An expired **T.vaginalis/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 **T.vaginalis/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

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the analysis. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

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

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

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

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by

the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

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

Emergency actions

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

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

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

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

Do not use the kit:

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

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

7. SAMPLES

The **T.vaginalis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from scrapes of epithelial cells from the urogenital tract.

General requirements

- PCR analysis is a direct method of laboratory analysis, and biological material sampling must be carried out from the site in the body where infectious process is localized. The decision to carry out the analysis should be taken by a consulting specialist based on anamnesis and the aspect of disease.
- The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
- In case of sampling from several biotopes repeat the procedure using new swabs and tubes for each biotope.
- Incorrect sampling may affect the results, in which case repeated sampling must be performed.
- During biomaterial preparation use RNase and DNase free filtered pipette tips.
- To avoid contamination only open the cap of the tube that is in work (adding sample/reagent, supernatant removal) and close it immediately afterwards.

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.

To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction.

Sample collection

WARNING! Before DNA extraction pre-processing of biological material samples may be needed.

Sample intake is made with special sterile single-use tools – probes, cytobrushes and swabs depending on the source of biological material according to established procedure.

WARNING!

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

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

The limitation of the method is the local use of medicines, transvaginal ultrasound less than 24 hours before the study.

The material is taken in accordance with the instructions for use to the NA extraction reagent kit.

Transport and storage of samples

Transport and storage conditions for scrapes of epithelial cells from urogenital tract are determined by instructions for use to the recommended DNA extraction reagent kits or transport media used for transport and storage.

Samples may be stored at temperatures from 2 °C to 8 °C for no longer 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 22 °C to minus 18 °C for one month (if it does not contradict to requirements of extraction kits used).

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

Biomaterial pretreatment (if necessary) is performed in accordance with the instruction for use to the NA extraction kit.

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-MB-RAPID**, **PREP-OPTIMA** and **PREP-MB-RAPID II** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

Table 4. Reagent kits recommended for DNA extraction for further analysis with **T.vaginalis/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	100
	PREP-GS-PLUS	300
PREP-MB	PREP-MB-RAPID	100
PREP-RAPID	PREP-RAPID	500
PREP-OPTIMA	PREP-OPTIMA, PREP-OPTIMA (shortened method in accordance with Annex B)	400
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 DNA extraction. 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. Preparing PCR for package S

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

WARNING! When using package S (R1-P126-S3/9EU), 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 with paraffin sealed PCR-mix for each test sample, negative control (C-) and positive control (C+).

WARNING! The amount 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 for samples, 1 tube for "C-" and 1 tube for "C+". The resulting number of tubes is 6.

8.1.2. Shake the tube with Taq-polymerase solution for 3-5 seconds on vortex mixer, then spin down for 1-3 seconds.

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

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

8.1.5. Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

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 spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. 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. Spin tubes/strips 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[3]. Add corresponding test[4], specify the number and IDs of the samples, negative and positive controls. Specify the position of the tubes/strips in the thermal unit (8.1.9) and run PCR. See Table 5.

For use with CFX96 (Bio-Rad) real-time thermal cycler, perform PCR with 35 µL of reaction mixture. See Table 6.

Table 5. The PCR program for DTlite and DTprime thermal cyclers for 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 6. Amplification program for CFX96 thermal cycles for 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, and Rox channels at 64 °C			

8.2. Preparing PCR for package U, manual dosing

WARNING!

1. For amplification, use 0.2 mL amplification tubes or 96-well sealed PCR microplates[5]. The use of strips is not recommended due to post-amplification contamination.
2. Reagents and tubes should be kept away from direct sunlight.

8.2.1 Mark the required number of 0.2 mL amplification tubes or a 96-well microplate for each test sample, 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).

[3] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.
[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.
[5] 96-well microplates are not used with DTlite detecting thermal cyclers

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 spin down for 1-3 seconds.

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 spin down for 1-3 seconds.

WARNING! Take TechnoTaq MAX polymerase out from the freezer 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

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 spin down for 1-3 seconds.

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 tube/well with PCR-mix. Cover the tubes loosely.

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 PCR-mix.

8.2.8 Shake the tubes with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes/wells 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 spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample. 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 microplate:

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

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:

Spin the tubes for 3-5 seconds in vortex mixer.

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

8.2.15 For DT thermal cyclers:

Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and ID's of the samples, negative and positive controls. Specify position of the tubes in thermal unit (see 8.2.12) and run PCR. The volume of reaction mixture is 18 µL. See Table 7.

For use with CFX96 (Bio-Rad) real-time thermal cyclers, perform PCR with 18 µL of reaction mixture. See Table 8.

Table 7. The PCR program for DTlite and DTprime thermal cyclers for package U

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

[6] Please, apply to Operation Manual for DTprime 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.

Table 8. Amplification program for CFX96 thermal cycles for 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, and Rox channels at 64 °C			

8.3. Preparing PCR for package U, using DTstream (only for DTprime thermal cyclers)

WARNING!

1. For amplification, use 384-well sealed PCR microplates.
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 spin down the drops for 1-3 seconds.

8.3.2 Shake the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex mixer, then spin down the drops for 1-3 seconds.

WARNING! TechnoTaq MAX polymerase should be got out from the freezer 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 spin down the drops for 1-3 seconds.

8.3.5 Shake the tube positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

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 spin down the drops for 1-3 seconds. DNA extraction kits, shake the tubes with DNA-preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.

8.3.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA sample preparations, positive and negative controls and PCR microplate on the DTstream working table and perform dispensing 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[8]. Add corresponding test[9], specify the number and ID's of the samples, negative and positive controls. Specify position of the tubes in thermal unit (see 8.3.10) and run PCR. See Table 7.

9. CONTROLS

The **T.vaginalis/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 **T.vaginalis/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 **T.vaginalis/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 Subtraction" tab choose "Baseline Subtraction Curve Fit".

10.3. Result interpretation is performed in accordance with Table 9. Run results are valid, if the result interpretation conditions for controls are observed.

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

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

Table 9. Interpretation of PCR results

Detection channel			**Result interpretation**
Fam, Cp/Cq	Hex, Cp/Cq	Rox, Cp/Cq	
Test samples			
Is specified	Is not considered	Is not specified	***Trichomonas vaginalis* DNA is detected**
Is not specified	Is not considered	**Is specified**	***Mycoplasma genitalium* DNA is detected**
Is not specified	**Is specified**	Is not specified	DNA of the sought microorganisms is not detected
Is not specified	Is not specified	Is not specified	Invalid result
Negative control			
Is not specified	**Is specified**	Is not specified	Negative result Run results are valid
Positive control			
Is specified	Is not considered	**Is specified**	Positive result Run results are valid

10.4. Unreliable result may be due to inhibitors in DNA preparation obtained from biological material; incorrect test 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 or Rox 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 **Trichomonas vaginalis 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 *Trichomonas vaginalis* 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 *Trichomonas vaginalis* or *Mycoplasma genitalium*, the detection thermal cycler software registers negative amplification results for the specific product and positive result for the internal control (IC).

ATTENTION! If the initial DNA concentration of one of the sought microorganisms is high, the false negative results is possible for the microorganism whose DNA concentration is low (see p.10. DATA ANALYSIS).

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: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *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 *Trichomonas vaginalis* 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 kit	Preparation volume, µL	Limit of detection, copies per sample
Scrapes of epithelial cells in 500 µL of transport medium	**PREP-NA**	50	50
	PREP-NA-PLUS	300	300
	PREP-GS	100	100
	PREP-GS-PLUS	300	300
	PREP-RAPID	500	500
	PREP-MB-RAPID[10]	100	100
	PREP-OPTIMA	400	400
	PREP-MB-RAPID II	100	100

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-NA-PLUS** (REF P-002/2EU), **PREP-GS** (REF P-003/1EU), **PREP-GS-PLUS** (REF P-003/2EU), **PREP-MB-RAPID** (REF P-116-N/4EU, REF P-116-A/8EU), **PREP-OPTIMA** (REF P-016-1/2EU, REF P-016-N/2EU) and **PREP-MB-RAPID II** (REF P-122-A/9EU, P-122-N/9EU, P-122-P/9EU, P-124-P/9EU) extraction kits.

c. Diagnostic characteristics

Target analyte	Diagnostic specificity (95CI)	Diagnostic sensitivity (95CI)
Trichomonas vaginalis	98.33% (91,06-99,96 %)	96.67% (82.78-99.92 %)
Mycoplasma genitalium	**98.46%** **(91.72-99.96 %)**	84.00% (63.92-95.46 %)

d. Repeatability

Total repeatability of results obtained using the kit is 100%.

[10] - only for scrapes of epithelial cells

12. TROUBLESHOOTING

Table 9. 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:

Phone: +7(495) 640.16.93

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:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

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

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 TO SYMBOLS

	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date		Catalogue number
	Batch code		Manufacturer
	Version		Keep away from sunlight
	Do not reuse		For research use only
	Non-sterile		Caution

Annex A

Shortened method of NA extraction from the test biomaterial (scrapes of epithelial cells from urogenital tract) using PREP-NA, PREP-NA-PLUS extraction kits

WARNING!

1. Before starting work:
   - switch on the thermostat and heat it 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).

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 reagent 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 buffer to the precipitate, shake the tubes on vortex for 3-5 seconds and spin down the drops 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 reaction mixture.

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 spin on vortex for 1-3 seconds.

DNA preparation is ready to be introduced into the reaction mixture.

WARNING! Only one thawing is allowed for DNA preparation!

Annex B

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

WARNING!

1. Before starting work, switch on the thermostat and heat 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. Spin down the drops 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 spin on vortex for 1-3 seconds.

DNA preparation is ready to be introduced into the reaction mixture.

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.

DNA preparation is ready to be introduced into the reaction mixture.

WARNING! Only one thawing is allowed for DNA preparation!