DNA-TECHNOLOGY

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For research use only

T.vaginalis/N.gonorrhoeae/C.trachomatis

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P111-S3/9ER
R1-P111-23/9ER
R1-P111-UA/9ER

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1. INTENDED USE

The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is intended for detection of *Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis* DNA in human biological material (urine, scrapes of epithelial cells from the urogenital tract) by real-time PCR.
Indications for the analysis: symptoms of infectious disease of the urogenital tract.

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

The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

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

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

2. METHOD

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 amplification reaction, the use of a hot-start is provided. For package S, hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin, 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 PCR-mix. 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) 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 *Neisseria gonorrhoeae* amplification product DNA includes the fluorescent dye Rox. The DNA probe used to detect *Chlamydia trachomatis* amplification product DNA includes the fluorescent dye Cy5. The DNA probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex.

Using several fluorescent dyes allows to reduce the number of tubes and the amount of biomaterial for the analysis as it is possible to register 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/Vic	Rox	Cy5	Cy5.5
Trichomonas vaginalis	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	-

The automatic analysis is available on "DNA-Technology" made instruments DTlite or DTprime REAL- TIME Thermal Cyclers for **T.vaginalis/N.gonorrhoeae/C.trachomatis 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.

The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

3. CONTENT

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

Table 2. The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P111-S3/9ER

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1920 µL (20 µL in each)	tubes, 12 strips of 8
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control*	Colorless transparent liquid	130 µL	1 tube
Strip caps	12 strips of 8		

* - marking as C+ is allowed

Table 3. The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P111-23/9ER

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1920 µL (20 µL in each)	96 tubes
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control*	Colorless transparent liquid	130 µL	1 tube

* - marking as C+ is allowed

Table 4. The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package U (standard), tubes for R1-P111-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*	Colorless transparent liquid	130 µL	1 tube

* - marking as C+ is allowed

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

The kit 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 package U is designed for 96 tests given at least 5 samples in a single run (3 test samples, negative and positive controls).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single-use sterile flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport media: “DNA-Technology” made **PREP-RAPID** (not applicable to male urethral smears), **STOR-F**, **STOP-M** or equivalent or sterile physiological saline solution 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) no less than 12,000);
- Solid-state thermostat (temperature range 50-65 °C);
- Tube rack for 1.5 mL tubes;
- RNase and DNase free 1.5 mL tubes (example, Eppendorf Safe-Lock Tubes);
- RNase and DNase free 0.2 mL PCR tubes with caps;
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-RAPID** (not applicable to male urethral smears), **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS, PREP-OPTIMA** and **PREP-MB RAPID** 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 0.2-1000 µL volume range);

- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Transport medium (if necessary);
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- DTstream dosing instrument (version 12M1 or 15M1, manufactured by "DNA-Technology R&P", LLC)[1];
- RNase and DNase free filter tips for DTstream *M1 (volume 200 µL) or those recommended for similar dosing instruments[1];
- DTpack plate sealing instrument (manufactured by "DNA-Technology R&P", LLC)[2];
- Centrifuge with microplate adapter, RCF(g) at least 100[2];
- Polymer microplate sealing thermal film[2];
- PCR microplate (only 96-well for manual dosing[3], only 384-well for automated dosing);
- 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.

[1] Package U, automated dosing
[2] If using microplates
[3] Shall not be used with DTlite thermal cycler

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit,** except TechnoTaq MAX polymerase (package U), shall be stored at temperatures from 2 °C to 8 °C throughout the shelf life of the kit. PCR-mix and paraffin sealed PCR-mix shall be stored at temperatures from 2 °C to 8 °C and out of light throughout the shelf life of the kit. TechnoTaq MAX polymerase shall be stored in a freezer at a temperature from minus 18 °C to minus 22 °C throughout the shelf life of the kit.

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

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

Transportation of the kit, except TechnoTaq MAX polymerase (package U), is allowed in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but for no longer than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport TechnoTaq MAX polymerase (package U) in thermobox with ice packs by all types of roofed transport at temperatures up to 25 °C but for no longer than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Kit transported with violation of temperature conditions shall not be used.

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

- components of the kit, except TechnoTaq MAX polymerase (package U), shall be stored at temperatures from 2 °C to 8 °C throughout the shelf life of the kit;
- TechnoTaq MAX polymerase (package U) shall be stored at temperatures from minus 18 °C to minus 22 °C throughout the shelf life of the kit;
- PCR-mix and paraffin sealed PCR-mix shall be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored under undue regime shall not be used.

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

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

The conformity of the **T.vaginalis/N.gonorrhoeae/C.trachomatis 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 diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. 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 the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

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

7. SAMPLES

The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from urine and scrapes of epithelial cells from the urogenital tract.

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.

The following substances are considered to be PCR inhibitors that may be present in the DNA sample: Hemoglobin and pharmaceuticals present in the DNA sample as a result of incomplete removal during DNA extraction from the biomaterial sample, isopropyl alcohol and methyl acetate remaining in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.

The maximum concentrations of interfering substances that have no effect on the amplification of the laboratory controls 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 assay (Miramistin®, chlorhexidine bigluconate).

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

General requirements

PCR analysis is a direct method, so taking of biological material must be carried out from the location of the infectious process.

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results.

Incorrect sample taking can lead to invalid results and the need for resampling.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

WARNING! Before DNA extraction pre-processing of samples is needed.

Sample collection

WARNING! Pretreatment, sampling and storage of the material is carried out in accordance with the instruction for use for DNA extraction kit.

Urine

The first portion of morning urine in the amount of 20–30 mL is selected for the analysis. The urine is taken into a special dry sterile container with volume of up to 60 mL, equipped with a hermetical screw-cap.

After the urine collection, container is tightly screwed and marked.

Scrapes from urogenital tracts

Sample taking 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. In case of pregnancy the use of cytobrushes for urogenital smears sampling is contraindicated.
2. Collect biomaterial into PREP-RAPID reagent using a dry swab! Avoid contact of reagent with skin, eyes and mucous membranes.
3. Before obtaining epithelial scrapes from urethra, posterior vaginal fornix and cervical canal, remove the free-flowing secretion 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.

Order of taking:

1 Open the tube with a transport medium.

2 After sample taking put the swab into the tube with transport medium and rinse it thoroughly for 10-15 seconds. Avoid spraying of solution.

3 Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.

4 Close the tube tightly and mark it.

If it is necessary to take biomaterial from several biotopes, repeat the procedure changing the swab and the tube each time.

N O T E - 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.

Transportation and storage of samples

Urine

Urine samples must be transported and stored:

- at temperature from 2 °C to 8 °C no longer than 1 day;
- at temperature from minus 18 °C to minus 22 °C no longer than one week.

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

Scrapes from urogenital tracts

Transportation and storage conditions for scrapes from urogenital tract are determined by the instructions for the transport media used for transportation and storage of the samples or by instructions for the recommended DNA extraction kits. (**PREP-RAPID**, **PREP-NA, PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS, PREP-OPTIMA** and **PREP-MB RAPID**).

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

Sample preparation

Biomaterial preparation (if necessary) is performed in accordance with instructions for use of the NA extraction kits.

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**, and **PREP-MB RAPID** extraction kits are recommended.

It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

WARNING! Independently of DNA extraction kit used, 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 sample in volumes as indicated.

8.1. PCR preparation for package S

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

WARNING! When using package S (R1-P111-S3/9EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1.1. Mark the required number of tubes with paraffin sealed PCR-mix for each test sample, negative control (C-) and positive control (C+).

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

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. Close the tubes.

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. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-OPTIMA** 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.
2. In case of using **PREP-GS and PREP-GS-PLUS** DNA extraction kits, shake the tubes with DNA preparation and negative control on vortex mixer for 3-5 seconds and centrifuge at RCF(g) 12,000–16,000 for one minute to precipitate the sorbent. 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.
3. In case of using **PREP-MB RAPID** extraction kit, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds to spin down the drops, 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 sample 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. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.8. Spin tubes/strips for 3-5 seconds on a vortex mixer.

8.1.9. Set the tubes/strips into the Real-time Thermal Cycler.

8.1.10. Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID’s of the samples, negative and positive control samples. Specify the position of the tubes/strips in the thermal unit and run PCR. See Table 5-7.

Table 5. The PCR program for DTlite and DTprime 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		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]		…	Holding		Holding

√ - optical measurement

[1] – holding at 25 °C is allowed

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

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

Table 6. The PCR program for CFX96 thermal cyclers (packages S, U)

Step	Temperature, °C	Time, min:sec	Cycle repeats
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 **√**	0:20	
√ - optical measurement (Plate Read), set the fluorescence measurement on the Fam, Hex, Rox and Cy5 channels at 64 °C			

Table 7. The PCR program for Applied Biosystems Quant Studio 5 thermal cyclers (packages S, U)

Stage	Step no.	Temperature, °C	Time, min:sec	Number of cycles (repeats)
Hold	1	80	01:00	1
	2	94	01:30	1
PCR stage	1	94	0:20	50
	2	64 **√**	0:20	
√ - data collection for fluorophores (Fam, Vic (Hex), Rox, Cy5) is on				

8.2. PCR preparation for package U, manual dosing

WARNING!

1. For amplification, use 0.2 mL amplification tubes or 96-well sealed PCR microplates[6] sealed with thermal film. The use of strips is not recommended due to post-amplification contamination.
2. Tubes with PCR-mix 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 analysis (3 test samples, negative and positive controls).

Example: to test 4 test samples, mark 4 tubes 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/microplate, 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,

[6] 96-well microplates are not used with DTlite detecting thermal cyclers

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

where N is the quantity of marked tubes/ necessary 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. 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 PCR-mix.

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

WARNING!

1. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-OPTIMA** 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.
2. In case of using **PREP-GS** and **PREP-GS PLUS** DNA extraction kits, shake the tubes with DNA preparation and negative control on vortex mixer for 3-5 seconds and centrifuge at RCF(g) 12,000–16,000 for one minute to precipitate the sorbent. 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.
3. In case of using **PREP-MB RAPID** extraction kit, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds to spin down the drops, 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/wells.

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 Carefully, without shaking 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 all the tubes/the microplate into real-time thermal cycler and proceed to PCR analysis (8.2.15, 8.2.16).

8.2.15 For DT thermal cyclers:

Launch the operating software for DT instrument[7]. Add corresponding test[8], specify the number and IDs 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 8.

8.2.16 For CFX (Bio-Rad) and QuantStudio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers:

Perform PCR analysis considering the 18 µL volume of reaction mixture according to tables 6, 7 respectively.

Table 8. 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	10[1]		…	Holding		Holding
√ - optical measurement [1] – holding at 25 °C is allowed						

8.3. PCR preparation for package U, automatic dosing using DTstream (only for DTprime thermal cyclers)

WARNING!

1. For amplification, use 384-well sealed PCR microplates.
2. Tubes with PCR-mix should be kept away from direct sunlight!

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

N o t e — It is recommended to test at least 5 samples per analysis (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. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-OPTIMA** 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.
2. In case of using **PREP-GS** and **PREP-GS PLUS** DNA extraction kits, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and centrifuge at RCF(g) 12,000–16,000 for one minute to precipitate the sorbent. 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.
3. In case of using **PREP-MB RAPID DNA Extraction Kit**, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds to spin down the drops, then put the tubes with the DNA preparation into magnetic rack. If, after DNA extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer 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 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 dispensing 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 with thermal film 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[9]. Add corresponding test[10], 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. The volume of reaction mixture is 18 µL. See Table 8.

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

9. CONTROLS

The **T.vaginalis/N.gonorrhoeae/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** contains positive control sample. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing.

The PCR-mix from the kit includes the internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance.

To reveal possible contamination a negative control is required.

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

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

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

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

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

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

10. DATA ANALYSIS

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

When the amplification program is complete, an information message will appear on the screen and you will be prompted to proceed to the analysis of the results. A graph will show the fluorescence vs. cycle number for all channels used for each assay in the thermoblock.

10.2 When using CFX96 (Bio-Rad) detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression) and choose “Baseline Subtraction Curve Fit” in the “Baseline Subtraction” tab.

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

Table 9. PCR results interpretation

Detection channel				Result interpretation
Fam, Cp/Cq/Ct	Hex/Vic, Cp/Cq/Ct	Rox, Cp/Cq/Ct	Cy5, Cp/Cq/Ct	
Test samples				
Is specified	Is not considered	Is not specified	Is not specified	***Trichomonas vaginalis*** **DNA is detected**
Is not specified	Is not considered	**Is specified**	Is not specified	***Neisseria gonorrhoeae*** **DNA is detected**
Is not specified	Is not considered	Is not specified	**Is specified**	***Chlamydia trachomatis*** **DNA is detected**
Is not specified	**Is specified**	Is not specified	Is not specified	DNA of the sought microorganisms is not detected
Is not specified	Is not specified	Is not specified	Is not specified	Invalid result*
Negative control				
Is not specified	**Is specified**	Is not specified	Is not specified	Negative result. The results are valid
Positive control				
Is specified	**Is specified**	**Is specified**	**Is specified**	Positive result. The results are valid

10.4 An invalid result can be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect performance of the analysis protocol; non-compliance with the amplification temperature regime, etc. In this case, PCR with the available DNA preparation needs to be repeated, or the DNA must be isolated and PCR performed again, or the biological material must be taken again (performed sequentially).

10.5 Cp/Cq/Ct values obtained for the biological sample on Fam, Rox or Cy5 detection channels less than 24 indicate the high concentration of the corresponding microorganism DNA. In this case a false-negative result is possible for the microorganism whose DNA is present in the low concentration. To eliminate the possibility of false-negative results, we recommend to repeat PCR of the extracted DNA preparation using "DNA-Technology" made **Trichomonas vaginalis REAL-TIME PCR Detection Kit, Neisseria gonorrhoeae REAL-TIME PCR Detection Kit and Chlamydia trachomatis REAL-TIME PCR Detection Kit.**

10.6 In case of results for negative control sample different from those in Table 7, the results of all series are considered invalid. In this case decontamination procedures are required.

10.7 In case of results for positive control sample different from those in Table 7, it is required to repeat amplification for all series.

11. SPECIFICATIONS

a. Analytical specificity

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

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

WARNING! If the initial DNA concentration of one of the sought microorganisms is high, the false negative results are 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: *Ureaplasma urealyticum*, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum, Mycoplasma genitalium, Candida albicans, Streptococcus sp., Staphylococcus sp*., 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, Neisseria gonorrhoeae* or *Chlamydia trachomatis* DNA in concentrations of up to 1.0×10^3 copies/mL of the sample.

b. Limit of detection (LOD)

LOD amounts to 5 copies of each microorganism DNA per amplification tube.

LOD is determined by the analysis of serial dilutions of the laboratory controls (LCs).

LOD in test sample depends on the used DNA extraction kit and the amount of the extracted DNA preparation obtained:

Biomaterial	Kits for DNA extraction	Volume of obtained preparation, µL	Limit of detection, copies/sample
Scrapes of epithelial cells in 500 µL of transport medium; urine (1.0 mL)	**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	300	300
	PREP-OPTIMA	400	400
*only for scrapes of epithelial cells			

c. Diagnostic characteristics

Biomaterial	**Target analyte**	**Diagnostic specificity**	**Diagnostic sensitivity**
Urine	*Trichomonas vaginalis*	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)
	Neisseria gonorrhoeae	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)
	Chlamydia trachomatis	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)
Scrapes of epithelial cells from urogenital tract	*Trichomonas vaginalis*	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)
	Neisseria gonorrhoeae	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)
	Chlamydia trachomatis	100% (95CI 86.3-100 %)	100% (95CI 86.3-100 %)

12. TROUBLESHOOTING

Table 10. Troubleshooting

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

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

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

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

13. QUALITY CONTROL

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

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

Technical support:

E-mail: hotline@dna-technology.ru

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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
	Non-sterile		