DNA-TECHNOLOGY

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For research use only

U.urealyticum+parvum/M.genitalium/C.trachomatis

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P113-S3/9EU
R1-P113-23/9EU
R1-P113-UA/9EU

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1. INTENDED USE

The **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** is intended for simultaneous detection of *Ureaplasma urealyticum* and *Ureaplasma parvum, Mycoplasma genitalium, 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 **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit.**

The **U.urealyticum+parvum/M.genitalium/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

Metod: 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 DNA-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. The polymerase chain reaction starts only when paraffin is melted.

Hot-start for package U is provided by using polymerase whose activity is blocked by antibodies, the activation of the enzyme occurs only after preheating the reaction mixture at 94 °C for 5 minutes.

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 *Ureaplasma urealyticum* and *Ureaplasma parvum* amplification product DNA includes the fluorescent dye Fam. The DNA probe used to detect *Mycoplasma genitalium* amplification product DNA includes the fluorescent dye Hex. 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 Rox. Table 1 shows the detection channels of amplification products.

Table 1– Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Ureaplasma urealyticum and *Ureaplasma parvum*	*Mycoplasma genitalium*	IC	*Chlamydia trachomatis*	-

The automatic analysis is available on "DNA-Technology" made instruments DTlite or DTprime REAL-TIME Thermal Cyclers for **U.urealyticum+parvum/M.genitalium/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.

3. CONTENT

The **The U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. **The U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P113-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	1920 µL (20 µL in each tube)	12 8-tube strips
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's caps	12 8-caps		

Table 3. The **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P113-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	1920 µL (20 µL in each tube)	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

Table 4. The **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** content, package U, for R1-P113-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Slightly 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

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 (package S) including no more than 94 experimental samples, negative control and positive control samples. The kit in the package U is intended for 96 samples and requires no less than 5 samples in a single run (3 experimental samples, positive and negative 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 **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU), **STOP-M** (REF P-910-1/1EU) 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 16000);
- 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);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, REF P-116-A/8EU) 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;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber (in case of using package U);
- Vortex mixer;
- Vortex rotor for 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;
- 0.2 mL PCR tubes (in case of using package U);
- 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 M1 dosage instrument (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips for DTstream dosage instrument (only for automated dosing in case of using package U ;
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- Centrifuge for microtrays (RCF(g) 500) (only for automated dosing in case of using package U);
- Polymer thermal seal for microtray sealing (only for automated dosing in case of using package U);
- PCR microtray (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. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** except TechnoTaq MAX polymerase (package U) must be stored at temperatures from 2 °C to 8 °C during the storage period.

TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

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

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

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

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

The kit stored in under undue regime should not be used.

An expired the **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit** should not be used.

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

The conformity of the **U.urealyticum+parvum/M.genitalium/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 **U.urealyticum+parvum/M.genitalium/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.

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

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) 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, and 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 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 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 decision about analyzing the location of sampling is done by a physician according to anamnesis and clinical picture.

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.

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

Sample collection

ATTENTION! Pretreatment, sampling and storage of the material is carried out in accordance with the user manual 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.

ATTENTION! In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

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 more than 1 day;
- At temperature from minus 18 °C to minus 22 °C no more than one week.

ATTENTION! Only one freezing-unfreezing 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-NA**, **PREP-GS**, **PREP-MB RAPID**)**.**

Sample preparation

It is necessary to perform pretreatment before DNA extraction by the **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** kits.

Urine

1. Transfer 1.0 mL of the sample to the 1.5 mL tube. Close the tubes tightly.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile saline solution to the precipitate. Close the tubes tightly.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Scrapes from urogenital tracts

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA**, **PREP-GS** 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.

ATTENTION! Independently of DNA extraction kit used, 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 as indicated.

Assay procedure

8.1 Preparing PCR for package S

ATTENTION! The reagents and tubes should be kept away from direct sun light.
ATTENTION! When using package S (R1-P113-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, positive control (C+) and negative 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 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.

8.1.5 Vortex the tubes with samples, “C+” and “C-“ for 3-5 seconds and spin down drops for 1-3 seconds.

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

In case of using **PREP-MB RAPID DNA Extraction kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds on a vortex mixer.

ATTENTION! Open the cap of 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 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[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.1.9) and run PCR. See Table 5.

Table 5. The PCR program for DTlite and DTprime Thermal Cyclers

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

8.2 Preparing PCR for package U, manual dosing

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

8.2.1 Mark the required number of 0.2 mL tubes for each test sample, positive control (C+) and negative 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.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.2.3 Add to each tube 6.0 µL of PCR-mix.

8.2.4 Vortex the PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

NOTE. TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

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

6.0 x (N+1) µL of PCR-buffer,
0.3 x (N+1) µL of TechnoTaq MAX polymerase,
N is a quantity of the samples to be tested taking to account "C-", "C+".

Example: for simultaneous testing of 4 samples, "C-" and "C+" in one PCR run, mark 6 tubes (4 tubes for samples to be tested, 1 tube for "C+" and 1 tube for "C-"). Prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

NOTE. Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to

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

use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube with PCR-mix.

NOTE. Follow the steps listed in pp. 8.2.8 – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

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

In case of using **PREP-MB RAPID DNA Extraction kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds on a vortex mixer.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes. 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 corresponding tube. Add 6.0 µL of positive control sample (C+) into corresponding tube.

8.2.11 Spin tubes for 3-5 seconds in a vortex mixer.

8.2.12 Set the tubes into the Real-time Thermal Cycler.

8.2.13 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.2.12) and run PCR. See Table 6.

Table 6. 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
[1] – holding at 25°C is allowed						

[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://dna-technology.com/assaylibrary.

8.3 Preparing PCR for package U, using DTStream

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

8.3.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.2 Vortex the TechnoTaq MAX polymerase and PCR-buffer for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

NOTE. TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.3.3 Prepare the mixture of PCR-buffer with TechnoTaq MAX polymerase according to the user manual for dosing device DTstream.

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

8.3.5 Vortex the tubes with DNA samples, “C-” and “C+” for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

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

ATTENTION! In case of using **PREP-MB RAPID DNA Extraction kit**, vortex the tubes for 3-5 seconds on a vortex mixer, put the tubes with the DNA preparation in magnetic rack and transfer the supernatant containing the isolated DNA to new tubes. If, after DNA extraction, the supernatant containing the isolated DNA was already transferred to new tubes, centrifugation is carried out for 3-5 seconds on a vortex mixer.

8.3.6 Put the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microtray on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microtray without shaking on the working table of DTpack sealing device.

8.3.8 Run the process of sealing of PCR microtray according to the user manual of DTpack sealing device.

8.3.9 Centrifuge the microtray at RCF(g) 500 for 30 seconds.

8.3.10 Put the PCR microtray into the thermoblock of detecting thermocycler.

8.3.11 Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.3.10) and run PCR. See Table 6.

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

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

9. CONTROLS

The **U.urealyticum+parvum/M.genitalium/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.

ATTENTION! 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 **U.urealyticum+parvum/M.genitalium/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 taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **U.urealyticum+parvum/M.genitalium/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 by inhibitors, operation error or violation of storage and handling.

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

10. DATA ANALYSIS

Registration of the 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.

When PCR is complete, the program displays the following indicators in the “Results” column of the table: indicator cycle (Cp) and the result for each assay (qualitative analysis).

The results are interpreted according to Table 7. The run results are valid if the conditions for interpreting the results obtained for the control samples are fulfilled.

Table 7. PCR results interpretation

Detection channel		Result	Result interpretation
Fam, Hex, Cy5	Rox		
Analyzed samples			
Cp is specified (for one or several channels)	Is not considered	+	DNA od one or several microorganisms (*Ureaplasma urealyticum* and *Ureaplasma parvum, Mycoplasma genitalium* or *Chlamydia trachomatis*) is detected
Cp is not specified (for all channels)	Cp is specified	-	DNA of the sought microorganisms is not detected
Cp is not specified (for all channels)	Cp is not specified	Invalid	Invalid result
Positive control sample			
Cp is specified (for all channels)	Cp is specified	+	Positive result. The results are valid
Negative control sample			
Cp is not specified (for all channels)	Cp is specified	-	Negative result. The results are valid

An unreliable 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).

Cp values obtained for the biological sample on Fam, Hex, 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 **Ureaplasma complex REAL-TIME PCR Detection Kit, Mycoplasma genitalium REAL-TIME PCR Detection Kit and Chlamydia trachomatis REAL-TIME PCR Detection Kit.**

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.

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 *Ureaplasma urealyticum* and *Ureaplasma parvum, Mycoplasma genitalium* or *Chlamydia trachomatis*, 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 *Ureaplasma urealyticum* and *Ureaplasma parvum, Mycoplasma genitalium* or *Chlamydia trachomatis*, 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: *Gardnerella vaginalis, Mycoplasma hominis, Neisseria gonorrhoeae, Candida albicans, Streptococcus sp., Staphylococcus sp*., and human DNA in concentration up to 1.0×10^8 copies/mL of the sample.

The absence of competitive inhibition has been shown in samples containing nonspecific DNA in high concentration and *Ureaplasma urealyticum* and *Ureaplasma parvum, Mycoplasma genitalium* or *Chlamydia trachomatis* DNA in low concentration.

b. Analytical sensitivity

Limit of detection amounts to 10 copies of each microorganism DNA per amplification tube.

Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

Limit of detection 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		
	PREP-NA/ 50	PREP-GS/ 100	PREP-MB RAPID/ 300
Scrapes of epithelial cells in 500 µL of transport medium; urine (1.0 mL)	100 copies/sample	200 copies/sample	600 copies/sample

c. Diagnostic characteristics

Biomaterial	**Microorganism DNA**	**Number of samples**	**Diagnostic sensitivity**	**Diagnostic specificity**
Urine	Chlamydia trachomatis	9	100% (66.37-100)	100% (97.42-100)
	Ureaplasma spp.	15	100% (78.20-100)	100% (97.30-100)
	Mycoplasma genitalium	12	100% (73.53-100)	100% (97.36-100)
Scrapes of epithelial cells from the urogenital tract	Chlamydia trachomatis	11	100% (71.51-100)	100% (97.39-100)
	Ureaplasma spp.	13	100% (75.29-100)	100% (97.34-100)
	Mycoplasma genitalium	11	100% (71.51-100)	100% (97.38-100)
All biomaterial	Chlamydia trachomatis	**20**	**100% (83.16-100)**	**100% (98.69-100)**
	Ureaplasma spp.	**28**	**100% (87.66-100)**	**100% (98.65-100)**
	Mycoplasma genitalium	**23**	**100% (85.18-100)**	**100% (98.68-100)**
Total	**71**	**100**	**100% (94.94-100)**	**100% (98.4-100)**

d. Within-batch and between-batch precision

Within-batch precision – 100% (83.16 – 100).

Between-batch precision – 100% (83.16 – 100).

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

12. TROUBLESHOOTING

Table 8. Troubleshooting

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

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

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

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

13. QUALITY CONTROL

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

- 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 **U.urealyticum+parvum/M.genitalium/C.trachomatis Multiplex REAL-TIME PCR Detection Kit**:

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
	Non-sterile		Caution