DNA-TECHNOLOGY

1470 2026-05-22

IVD

For professional use only

Ureaplasma parvum REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P105-S3/9EU
R1-P105-23/9EU
R1-P105-UA/9EU

Version: 1470.2026.05.22

TABLE OF CONTENTS

1. INTENDED USE

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Ureaplasma parvum REAL-TIME PCR Detection Kit** is designed to detect *Ureaplasma parvum* DNA in human biological samples (epithelial cell swabs from urogenital tract, urine, prostate fluid, ejaculate) by real-time PCR.

Functional purpose: *in vitro* diagnostics.

Indications for use: symptoms of infectious or inflammatory diseases of the urogenital tract, control of the treatment of infection caused by *Ureaplasma parvum*.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Ureaplasma parvum REAL-TIME PCR Detection Kit.**

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: qualified personnel trained in molecular diagnostic methods.

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

2. METHOD

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

The implemented PCR method is based on amplification of a target DNA sequence. The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent elongation of the polynucleotide chains from these primers with Taq polymerase.

To increase sensitivity and specificity of the 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. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures. “Hot” start for package U is provided by using polymerase which activity is blocked by antibodies. The activation of the enzyme occurs only after preheating the reaction mixture at 94°C. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures.

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

PCR mix includes internal control (IC) designed to control the quality of polymerase chain reaction.

The DNA probe used to detect the *Ureaplasma parvum* amplification product includes fluorescent dye Fam. The DNA probe used to detect the IC amplification product includes fluorescent dye Hex.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Ureaplasma parvum	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite, DTprime or DTprime II real-time thermal cyclers for **Ureaplasma parvum 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 **Ureaplasma parvum REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad), Rotor-Gene Q (Qiagen), and Applied Biosystems QuantStudio 5 (Life Technologies Pte. Ltd.) real-time thermal cyclers.

3. CONTENT

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** comes in package S (tubes/strips) and package U. The detailed description of content is represented in Tables 2–4.

Table 2. The **Ureaplasma parvum REAL-TIME PCR Detection Kit** content, package S (strips) for R1-P105-S3/9EU

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

Table 3. The **Ureaplasma parvum REAL-TIME PCR Detection Kit** content, package S (tubes) for R1-P105-23/9EU

Reagent	Description	Amount	Volume per tube
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	96 individual tubes	20 µL in each
Taq polymerase solution	Colorless transparent liquid	2 tubes	500 µL in each
Mineral oil	Colorless transparent viscous oily liquid	2 tubes	1.0 mL in each
Positive control[1]	Colorless transparent liquid	1 tube	130 µL

Table 4. The **Ureaplasma parvum REAL-TIME PCR Detection Kit** content, package U for R1-P105-UA/9EU

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

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

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** in package S is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** in package U is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

[1] - marking as C+ is allowed

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

The following equipment, reagents and consumables are required:

Reagents, equipment and consumables	Package S		Package U, dosing	
	strips[1]	tubes	manual	automated
UV PCR cabinet	•	•	•	•
Real-time detecting thermal cycler[2]	•	•	•	•[3]
Vortex mixer	•	•	•	•
Vortex rotor for 0.2 mL strips	•	-	-	-
Refrigerator with freezer	•	•	•	•
Tube rack for 0.2 mL tubes	-	•	•	-
Tube rack for 0.2 mL strip tubes	•	-	-	-
Tube rack for 1.5 mL tubes	•	•	•	•
Single channel pipettes (dispensers covering 2.0–20 µL; 20–200 µL; 200–1,000 µL volume range)	•	•	•	•
RNase and DNase free filtered pipette tips (volume 20 µL)	•	•	•	•
RNase and DNase free pipette tips (volume 200 µL; 1,000 µL)	•	•	•	•
Pipette rack	•	•	•	•
RNase and DNase free 1.5 mL microfuge tubes with caps	•	•	•	•
RNase and DNase free 0.2 mL PCR tubes with caps	-	-	•	-
Powder-free surgical gloves	•	•	•	•
Container for used pipette tips, tubes and other consumables	•	•	•	•
DTstream12 M1 or DTstream15 M1 liquid handling station	-	-	-	•
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream* M1, or similar	-	-	-	•
DTpack plate sealing device	-	-	-	•
Centrifuge for microplates (RCF(g) at least 100)	-	-	-	•
Polymer thermal film for microplate sealing	-	-	-	•
96-well PCR microplate	-	-	•	•
384-well PCR microplate	-	-	-	•
Physiological saline solution 0.9% NaCl (sterile) (if necessary)				
Transport medium (if necessary), the following are recommended: – **STOR-F** transport medium for biomaterial samples – **STOR-M** transport medium for epithelial cell swabs from urogenital tract				
NA extraction reagent kits[4], the following are recommended: – **PREP-NA;** – **PREP-GS**; – **PREP-RAPID**; – **PREP-OPTIMA**; – **PREP-MB-RAPID II**.				
Notes and specifications: [1] – not used with Rotor-Gene Q detecting thermal cycler [2] - hereinafter – detecting thermal cycler; the required parameters are indicated below [3] - DTprime *X* and DTprime II *X* are validated [4] - possibility of DNA extraction reagent kit/set use for extraction of target microorganisms' DNA is determined by the biomaterial type (see 8.1) "•" – the piece of equipment/reagent is required "–" – the piece of equipment/reagent is not required				

The following detecting thermal cyclers are validated for work with the **Ureaplasma parvum REAL-TIME PCR Detection Kit**:

- DTprime in DTprime *M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime;
- DTprime II in DTprime II *M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II;
- DTprime in DTprime *X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime *X*(only for package U, automatic dosing using 384-well PCR microplates);
- DTprime II in DTprime II *X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II *X* (only for package U, automatic dosing using 384-well PCR microplates);
- DTlite in DTlite *S* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTlite (only for package S and package U, manual dosing, tubes);
- Rotor-Gene Q (manufactured by QIAGEN GmbH, Germany), hereinafter – Rotor-Gene Q (only for package S, tubes, and package U, manual dosing, tubes);
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96;
- Applied Biosystems QuantStudio 5 (manufactured by Life Technologies Pte. Ltd., Singapore), hereinafter – Applied Biosystems QuantStudio 5.

For the use of detecting thermal cyclers other than those listed in the table, please consult the reagent kit manufacturer.

Software:

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Storage conditions

5.1.1. Package S

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

5.1.2. Package U

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

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

5.2. Transport conditions

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

5.2.1. **Package S**

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

5.2.2. **Package U**

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

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

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

5.3.1. **Package S**

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

5.3.2. **Package U**

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

WARNING! The kits stored under undue regime must not be used.

An expired **Ureaplasma parvum 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.

Conformity of **Ureaplasma parvum 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

- For *in vitro* diagnostic use only.
- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or invalid results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.

- Avoid any direct contact with the biological samples, reagents and materials used to carry out the test. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- Samples must be handled under a laminar flow hood.
- Pipettes used to handle samples must only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips.
- The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as if infectious[2, 3]. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 min or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 min before and after the assay. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

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

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

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

Do not use the kit:

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

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

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

- after the expiry date of the kit.

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

7. SAMPLES

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the urogenital tract, urine, prostate fluid, ejaculate.

7.1. General requirements

PCR analysis refers to direct methods of laboratory research; therefore, the collection of biological material must be carried out from the site of infection localization. The decision to examine a localization shall be taken by a physician based on the collected anamnesis and the clinical picture of the disease.

The quality of sampling, sample storage, transport, and pretreatment are of great importance for obtaining correct results.

Incorrect sampling may lead to invalid results and, therefore, to the necessity for repeated sampling.

Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.

To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.

7.2. Interfering substances

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

PCR inhibitors are: hemoglobin present in DNA sample due to incomplete removal of blood impurities from the DNA sample during DNA extraction; isopropyl alcohol and methyl acetate present in DNA sample due to incomplete removal of wash solutions during sample preparation.

Maximum concentrations of interfering substances not affecting the amplification of laboratory control and IC: hemoglobin – 0.35 mg/mL of DNA sample; isopropyl alcohol – 100 µL/mL of DNA sample; methyl acetate – 100 µL/mL of DNA sample.

Lubricants and local medicines in DNA sample may also affect the PCR results. To reduce the count of PCR inhibitors, it is necessary to follow the principles of biomaterial intake. 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.

7.3. Sample collection

WARNING! Sample preparation may be required before DNA extraction!

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

Method limitations[4]: local application of medicines, lubricants, vaginal ultrasound, colposcopy less than 24 hours before the test.

The taking of swabs is carried out:

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

[4] - If it does not contradict the requirements of the NA extraction kit.

Note. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

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

Biomaterial collection is carried out in accordance with instructions for use to the NA extraction kits.

7.3.1.1 Urogenital sampling

Women should not carry out genitals' toilet and vaginal douching the day before research. To obtain an accurate result, it is necessary that the material contains the largest possible count of epithelial cells and the minimum amount of mucus and blood impurities.

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

In the case of taking biomaterial from several biotopes, repeat the procedure, taking the material with a new swab into a new tube each time.

7.3.1.2 Vaginal sampling

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

7.3.1.3 Urethral sampling

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

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

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

7.3.1.4 Cervical sampling

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

7.3.2 First portion of morning urine

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

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

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

After urine collection, container is tightly screwed and marked.

7.3.3 Prostate fluid

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

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

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

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

The container with prostate fluid is hermetically screwed and marked.

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

7.3.4 Ejaculate

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

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

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

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

The container with ejaculate is hermetically closed and marked.

7.4. Transport and storage of samples

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples (see 8.1).

Samples may be transported and stored in physiological saline at temperatures from 2°C to 8°C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the 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 the requirements to the used NA extraction reagent kits/sets).

WARNING! Avoid repeated freezing and thawing of samples.

7.5. Biomaterial preparation for DNA extraction

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

8. PROCEDURE

8.1 DNA extraction from biological material

NA extraction is performed in accordance with the instructions for use of the corresponding reagent kit.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA, PREP-GS, PREP-RAPID, PREP-OPTIMA,** and **PREP-MB-RAPID II** extraction kits are recommended.

Note. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

WARNING! Run a negative control alongside DNA extraction through all preparation stages. Use physiological saline solution (in volumes per the extraction kit instructions) or the kit provided negative control.

General requirements:

- Follow the DNA preparation recommendations from the NA extraction kit's instruction.
- Use filter tips.
- Open tube caps only when introducing the DNA sample, then close immediately.
- If using strips: close the strip cap after introducing samples before proceeding to the next strip.
- Ensure all tubes are tightly closed after dosing.

For DNA preparations after storage, prepare them as follows before adding to the amplification mixture:

- **PREP-NA, PREP-RAPID, PREP-OPTIMA**: vortex (3–5 sec), then centrifuge on vortex (1–3 sec).
- **PREP-GS**: vortex (5–10 sec), incubate at 50 °C (5 min) (if the eluate was refrigerated), then centrifuge at 16,000 RCF(g) (1 min).
- **PREP-MB-RAPID II**: centrifuge on vortex carefully (without shaking, 1–3 sec), place into magnetic rack. If supernatant transferred to new tubes — centrifuge (1–3 sec) after shaking.

8.2 Preparing PCR for package S

WARNING!

- The reagents and tubes should be kept away from direct sunlight.
- When using package S, strips, strictly observe the completeness of the strips and caps. Do not use the caps for the strips of the other kits!

8.2.1 Mark one tube/strip tube with the paraffin-sealed PCR mix for each test sample, C-, and C+.

WARNING! The volume of reagents is calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 4 samples, mark 4 tubes for samples, one C- tube and one C+ tube. Total number of tubes is 6.

8.2.2 Shake the tubes with Taq polymerase solution on vortex, then spin down the drops.

8.2.3 Add **10 µL** of Taq polymerase solution to each tube. Avoid paraffin layer break.

WARNING! If using Rotor-Gene Q detecting thermal cycler, do not add mineral oil into the tubes.

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

8.2.5 Shake the tube with C+ on vortex, then spin down the drops.

8.2.6 Add **5.0 µL** of DNA sample into corresponding tubes. Do not add DNA into the C- and C+ tubes. Avoid paraffin layer break.

8.2.7 Add **5.0 µL** of C- which passed whole DNA extraction procedure into the C- tube. Avoid paraffin layer break.

8.2.8 Add **5.0 µL** of C+ into the corresponding tube. Avoid paraffin layer break.

8.2.9 Thoroughly spin down the drops on vortex (if using Rotor-Gene Q detecting thermal cycler, spinning is not required).

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

8.2.11 **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/strips in thermal unit (see 8.2.10) and run PCR. See Table 5.

8.2.12 **For Rotor-Gene Q, CFX96, Applied Biosystems QuantStudio 5 detecting thermal cyclers:**

Perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 6, 7, 8, respectively.

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

[6] - The test for DT detecting thermal cyclers is created by entering parameters (indicated in Annex A) or is provided by the kit manufacturer.

Table 5. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (package S)

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[7]	…	…	Holding		Holding
√ – optical measurement						

Table 6. The PCR program for Rotor-Gene Q thermal cyclers (package S, tubes)

Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	90	
Cycling 2	94 deg	30	5 times
	57 deg √	15	
Cycling 3	94 deg	10	45 times
	57 deg √	15	
√–optical measurements, set the fluorescence measurement (Acquiring) on the Green (Fam), and Yellow (Hex) channels at 57°C			

Table 7. The PCR program for CFX96 thermal cyclers (packages S, U)

Step	Temperature, °C	Time, min:sec	Number of cycles
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ – optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex channels at 64°C			

Table 8. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

Stage	Step	Temperature, °C	Time, min:sec	Number of cycles
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	0:20	50
	2	64 **√**	0:20	
√ – data collection for the necessary fluorophores (Fam, Vic (Hex)) is on				

[7] - Holding at 10°C is allowed.

8.3 Preparing PCR for package U, manual dosing

WARNING!

- The reagents and tubes must be kept away from direct sunlight.
- For amplification use 0.2 mL single-use amplification tubes or 96-well PCR plates[8], sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.

8.3.1 **Mark** the required number of 0.2 mL tubes or a 96-well plate for each test sample, C- and C+.

Note. It is recommended to test at least 5 samples per test (3 test samples, C- and C+).

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

8.3.2 Shake the tubes with PCR mix on vortex, then spin down the drops.

8.3.3 Add **6.0 µL** of PCR mix to each marked tube/well (including C- and C+).

8.3.4 Shake tubes with PCR buffer and TechnoTaq MAX polymerase on vortex, spin down the drops.

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

8.3.5 Prepare the mix 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,

where N is the quantity of samples to be tested taking into account C-, C+.

Example: to test 4 samples, C- and C+ in one PCR run, mark 6 tubes/reserve 6 wells (4 tubes/wells for test samples, 1 tube/well for C- and 1 tube/well for C+). Prepare the mix of PCR buffer and Taq polymerase for 7 (6+1) tubes/wells. Mix 42 µL of PCR buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.3.6 Vortex the tubes with the mix of PCR buffer and TechnoTaq MAX and spin down the drops.

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

8.3.7 Add **6.0 µL** of the mix of PCR buffer and TechnoTaq MAX polymerase 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 the mix of PCR buffer and TechnoTaq MAX polymerase to PCR mix.

8.3.8 Shake the tubes with C+ on vortex, then spin down the drops.

8.3.9 Add **6.0 µL** of DNA sample into corresponding tubes/wells. Do not add DNA into the C-, C+ tubes/wells.

8.3.10 Add **6.0 µL** of C- which passed whole DNA extraction procedure into the corresponding tube/well.

8.3.11 Add **6.0 µL** of C+ into the corresponding tube/well.

8.3.12 In case of using **96-well PCR microplates**:

8.2.12.1. Place the plate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR plate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at **RCF(g) 100 for 30 sec**.

8.3.13 In case of using **tubes**:

Centrifuge the tubes for 3–5 sec on vortex mixer (if using Rotor-Gene Q detecting thermal cycler, centrifugation is not required).

[8] - 96-well plates are not used with DTlite and Rotor-Gene Q detecting thermal cyclers

8.3.14 Set the tubes/microplate into the real-time thermal cycler.

8.3.15 **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/microplate in thermal unit (see 8.3.13) and run PCR. See Table 9.

8.3.16 **For CFX96, Applied Biosystems QuantStudio 5, Rotor-Gene Q detecting thermal cyclers:**

Perform PCR considering reaction mixture volume of 18 µL according to amplification programs shown in Tables 7, 8, 10, respectively.

Table 9. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (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[11]			Holding		Holding
√ – optical measurements						

Table 10. The PCR program for Rotor-Gene Q thermal cycler (package U)

Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	300	
Cycling 2	94 deg	30	5 times
	57 deg √	15	
Cycling 3	94 deg	10	45 times
	57 deg √	15	
√ – optical measurements, set the fluorescence measurement (Acquiring) on the Green (Fam), and Yellow (Hex) channels at 57°C			

8.4 Preparing PCR for package U, using DTstream

WARNING!

- The reagents and tubes should be kept away from direct sunlight.
- For amplification use 96-well or 384-well[12] PCR microplates hermetically sealed with thermal film.

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

[10] - The test for DT detecting thermal cyclers is created by entering parameters (indicated in Annex B) or is provided by the kit manufacturer.

[11] - Holding at 10 °C is allowed.

[12] - Only for DTprime *X* and DTprime II *X* detecting thermal cyclers.

Note – It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.4.1 Shake the tube with PCR mix on vortex, then spin down the drops.

8.4.2 Shake the tube with PCR buffer and TechnoTaq MAX polymerase on vortex, then spin down the drops.

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

8.4.3 Prepare the mix of PCR buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.4.4 Shake the tube with the mix of PCR buffer and TechnoTaq MAX polymerase on vortex, then spin down the drops.

8.4.5 Shake the tubes with C+ on vortex, then spin down the drops.

8.4.6 Set the tubes with PCR mix, the mix of PCR buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls, and PCR microplate on the DTstream working table and **conduct dosage** of the components according to DTstream user manual.

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

8.4.8 Seal the PCR microplate according to the user manual of DTpack sealing device.

8.4.9 Centrifuge the microplate at **RCF(g) 100 for 30 sec**.

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

8.4.11 **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument[13]. Add corresponding test[14], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/plates in thermal unit (see 8.4.10) and run PCR. See Table 9.

8.4.12 **For CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers:**

Perform PCR considering reaction mixture volume of 18 µL according to amplification programs shown in Tables 7, 8, respectively.

9. CONTROLS

The **Ureaplasma parvum REAL-TIME PCR Detection Kit** contains positive control. Positive control is a cloned part of the *Ureaplasma parvum* genome. 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 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 **Ureaplasma parvum 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 absence and for internal control is present.

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

[14] - the test for DT detecting thermal cyclers is created by entering parameters (indicated in Annex B) or is provided by the kit manufacturer.

For **Ureaplasma parvum 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

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

10.2 When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the "Baseline Setting" tab select "Baseline Subtraction Curve Fit".

10.3 Result interpretation is carried out according to Table 11. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 11. PCR results interpretation

Fam/Green (target DNA), Cp/Cq/Ct	**Hex/Yellow/Vic** (IC), Cp/Cq/Ct	**Result interpretation**
Test samples		
Specified	Not considered	***Ureaplasma parvum* DNA is detected**
Not specified	**Specified**	*Ureaplasma parvum* DNA is not detected
Not specified	Not specified	Invalid result
Negative control		
Not specified	**Specified**	**Negative result** Run results are valid
Positive control		
Specified	Not considered	**Positive result** Run results are valid

10.4 Invalid result may be due to the presence of inhibitors in the NA preparation obtained from biological material; incorrect execution of the analysis protocol, noncompliance with the amplification temperature regime, etc. In this case it is necessary to repeat PCR with the available DNA preparation, or to re-extract DNA and perform PCR for this sample, or to re-collect biological material from the patient (performed sequentially).

10.5 If a positive result is obtained for C-, the results of the whole batch should be considered invalid. In this case special measures are required for detection and elimination of possible contamination.

10.6 If a negative result is obtained for C+, the results of the whole batch should be considered invalid. In this case it is required to repeat PCR for the whole batch of samples.

11. SPECIFICATIONS

a. Analytical specificity

For human biomaterial samples containing DNA of *Ureaplasma parvum*, the detecting thermal cycler software registers positive result of specific product (*Ureaplasma parvum* genome fragment) amplification on Fam/Green detection channel.

For human biomaterial samples not containing DNA of *Ureaplasma parvum*, the detecting thermal cycler software registers negative result of specific product (*Ureaplasma parvum* genome fragment)

amplification on Fam/Green detection channel and positive result of IC amplification on Hex/Yellow/Vic detection channel.

No non-specific amplification results were observed in the presence of *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Trihomonas vaginalis, Streptococcus* spp., *Staphylococcus* spp. DNA, and human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity (limit of detection)

Limit of detection (LoD) is 10 copies of *Ureaplasma parvum* DNA per amplification tube. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS). 94 tests were made for each concentration.

The concentration of LCS, copies per amplification tube	Number of repetitions	Number of positive results	% of positive results
20	94	94	100
10	94	94	100
5	94	80	85.1
2	94	62	65.9
0	94	0	0

Sensitivity of *Ureaplasma parvum* DNA in the sample depends on the sampling and the final volume of the extracted DNA (elution volume).

Sensitivity of 10 copies per amplification tube corresponds to the following values of the DNA concentration of *Ureaplasma parvum* in case of using DNA extraction kits produced by DNA-Technology:

Biomaterial	**PREP-NA**	**PREP-GS**	**PREP-RAPID**	**PREP-OPTIMA**	**PREP-MB-RAPID II (elution 100 µL)**
– Epithelial cell swab in 500 µL of transport medium; – Ejaculate in 500 µL of transport medium; – Prostate fluid (extraction from 100 µL of sample); – Urine (extraction from 1.0 mL of sample)	100 copies/ sample	200 copies/ sample	1,000 copies/ sample	800 copies/ sample	200 copies/ sample

c. Diagnostic characteristics

Number of samples (n) - 298;

Diagnostic sensitivity (95% CI) – 97.9% (91.1-99.8%);

Diagnostic specificity (95% CI) – 99.6% (98.3-100%).

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

12. TROUBLESHOOTING

Table 12. 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 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 control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our customer service with quality issues of **Ureaplasma parvum 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, Serpukhov Urban District,

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Phone/fax: +7(495) 640.17.71

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https://www.dna-technology.com

14. KEY SYMBOLS

IVD	*In vitro* diagnostic medical device		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
	Non-sterile		Do not reuse

Annex A

Test parameters for DTprime, DTprime II, DTlite detecting thermal cyclers for package S

1) Number of tubes in the test – 1;

2) PCR mix volume – 35 µL;

3) Enter the following parameters in the “Amplification program” window:

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[15]	…	…	Holding		Holding
√ - optical measurements						

4) Enter the following detection channel parameters:

Fam	Hex	Rox	Cy5	Cy5.5
Ureaplasma parvum	IC	-	-	-

[15] - holding at 10°C is allowed

Annex B

Test parameters for DTprime, DTprime II, DTlite detecting thermal cyclers for package U

1) Number of tubes in the test – 1;

2) PCR mix volume – 18 µL;

3) Enter the following parameters in the “Amplification program” window:

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[16]	…	…	Holding		Holding
√ - optical measurements						

4) Enter the following detection channel parameters:

Fam	Hex	Rox	Cy5	Cy5.5
Ureaplasma parvum	IC	-	-	-

[16] - holding at 10°C is allowed