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For professional use only

Chlamydia trachomatis REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – for qualitative pathogen-detection. The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is designed to detect *Chlamydia trachomatis* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: urine, prostate fluid, ejaculate, scrapings of epithelial cells (from the urogenital tract, oropharynx, rectum, conjunctiva of the eye).

The most common chlamydial infection affects the organs of the reproductive system. Chlamydial infection can occur both with clinical symptoms and asymptomatic, but regardless of the presence of symptoms, chlamydial infection causes serious complications in the form of reproductive disorders and the development of male and female infertility. Infection transmitted during sexual intercourse or at the birth of a child through an infected genital tract.

Long-term current, undetected and untreated infection in women can lead to the fact that bacteria penetrate through the uterus into the upper genital tract, ultimately, lead to such complications as pelvic inflammation, ectopic pregnancy and tubal infertility.

Complications of chlamydial infection in men may be epididymitis, epididymo-orchitis and sexually acquired reactive arthritis. Chlamydial infection in men is also associated with reduced reproductive capacity or infertility as a result of direct effects on sperm: its maturation, mobility and viability.

More rarely chlamydial infection affects the rectum, oropharynx, conjunctiva of the eyes.

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

The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Chlamydia trachomatis* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of two fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. 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
Chlamydia trachomatis	IC	-	-	-

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers (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 **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) real-time thermal cyclers.

3. CONTENT

The detailed description of content is represented in the Tables 2.1 - 2.2.

Table 2.1. The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P101-23/9EU and R1-P101-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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 [1]	12 8-caps		

Table 2.2. The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** content, package U (universal) for R1-P101-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or 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 **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (no more than 94 defined samples, one positive control and one negative control) for package S.

Package U is designed to carry out 96 tests if at least 5 samples in one study are amplified (3 test samples, positive and negative control samples)

.

[1] - for detection kit packaged in strips REF R1-P101-S3/9EU

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

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

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID** REF P-001/1EU (not applicable to male urethral swabs), **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- 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 **Chlamydia trachomatis REAL-TIME PCR Detection Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °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 TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

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

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport TechnoTaq MAX polymerase at temperatures from 2 °C to 8 °C for no more than 5 days.

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

The kit stored under undue regime should not be used.

An expired **Chlamydia trachomatis 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 **Chlamydia trachomatis REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the ***Chlamydia trachomatis*** **REAL-TIME PCR Detection Kit**.

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, reverse transcription, 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 breach;
- 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 instruction for use.

7. SAMPLES

The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from urine, prostate fluid, ejaculate, scrapings of epithelial cells (from the urogenital tract, oropharynx, rectum, conjunctiva of the eye), depending on professional prescription.

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 amplification.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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.

Following medicines have no effect on the amplification of the laboratory control sample and internal control: chlorhexidine bigluconate – 5.0%, Miramistin ® - 5.0%.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra), rectum swabs sampling

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

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

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

 Samples may be stored at temperatures from 2 °C to 8 °C no more than 24 hoursprior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

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

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1 The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25°C.

NOTE - Use a centrifuge for 1.5 mL tubes with RCF not less RCF(g) 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000)

4.2 Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

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

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Residual urine after prostate massage

Before residual urine after prostate massage, sexual abstinence is recommended for 3 days before the examination.

The patient urinates in the toilet, leaving part of the urine in the bladder.

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

The prostate massage is carried out for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: with a soft prostate - slight pressure is carried out, with a dense consistency of the prostate - the pressure force is increased.

After the end of the massage, the first 10-15 mL of the urine is collected in a sterile container with a volume of up to 60 mL.

Container is tightly screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month

NOTE - The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS**, and **PREP-MB RAPID** extraction kits instructions for use.

8. PROCEDURE

DNA extracting from biological material.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from men urogenital swabs.

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

Assay procedure

8.1 Preparing PCR for package S

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

When using package S, strips, strictly observe the completeness of the strips and caps for them. Do not use caps for strips from other kits!

8.1.1. Mark tubes with 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 Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.1.4. Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes.

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

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 in a vortex mixer.

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

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 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-" and "C+" tubes. Avoid paraffin layer break.

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

8.1.8. Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene thermal cycler, centrifugation is not required).

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

8.1.10. Launch the operating software for DT instrument[2]. Add corresponding test[3], 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 Tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

NOTE - Amplification products can be stored at temperatures from 2 °C to 8 °C for one month or at a temperature minus 20 °C for 12 months.

8.2 Preparing PCR for package U

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 sample to be tested, for positive control (C+) and for negative control (C-).

Example: to test 4 samples in one PCR run, 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 and spin down drops for 1-3 seconds.

8.2.3. Add 6.0 µL of PCR-mix into the each marked tube for samples to be tested.

8.2.4. Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin for down drops for 1-3 seconds.

TechnoTaq MAX polymerase must be stored at temperatures from minus 18°C to minus 22°C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.2.5. Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase.

Add into one tube:

- 6.0×(N+1) µL of PCR-buffer,
- 0.3×(N+1) µL of TechnoTaq MAX polymerase,

N — number of the marked tubes including "C-" and "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 TechnoTaq MAX polymerase for 7 (6+1) tubes. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

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

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

8.2.6. Vortex the tube with prepared mixture for 3-5 seconds, then spin down drops for 1-3 seconds.

The mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared just prior to use.

8.2.7. Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each PCR-tube. Close the tubes.

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 amplification mix.

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

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 in a vortex mixer.

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

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

8.2.9. Add 6.0 µL of DNA sample into corresponding PCR-tubes. Do not add DNA into the “C-” and “C+” tubes.

8.2.10. Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-” tube and positive control (C+) into corresponding tube.

8.2.11. Spin tubes for 3-5 seconds.

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

8.2.13. Launch the operating software for DT instrument[4]. Add corresponding test[5], 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 Tables 7-11.

8.3 Preparing PCR for package U using DTstream

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 and spin down drops for 1-3 seconds.

8.3.2. Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin down drops for 1-3 seconds.

TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.3.3. Following the DTstream software instructions, prepare a mixture of PCR-buffer with TechnoTaq MAX polymerase in a separate test tube.

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

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

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

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 in a vortex mixer.

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. Set tubes with PCR-mix, PCR-buffer and TechnoTaq MAX polymerase mixture, DNA sample, positive control and negative control and microplate for PCR to the DTstream and dispense the components according to the user manual.

8.3.7. After completion of the program on the DTstream, set gently, without shaking, the microplate to the DTpack.

8.3.8. Carry out the procedure of sealing the microplate by thermal film in accordance with the instructions to the DTpack.

8.3.9. Spin the microplate at RCF(g) 1000 for 30 seconds.

8.3.10. Set the microplate into the Real-time Thermal Cycler.

8.3.11. Launch the operating software for DT instrument[6]. Add corresponding test[7], 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 Tables 7-11.

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

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

Table 3. 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
√ - optical measurement [1] – holding at 25°C is allowed						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	Cy5.5/Crimson
Specific product and C+	IC	-	-	-

Table 8. 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	5	15		Cycle
	94	0	5			
2	94	5	0	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						

Table 9. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	5 min	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 10. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	5 min	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 11. The PCR program for Rotor-Gene thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

9. CONTROLS

The **Chlamydia trachomatis REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Chlamydia trachomatis* 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.

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

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.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control are 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers the analysis performed automatically. In other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Chlamydia trachomatis* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Chlamydia trachomatis* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current instruction for use). The cutoff Ct values for Rotor-Gene thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical **specificity** of the **Chlamydia trachomatis REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Chlamydia trachomatis* DNA are to be registered positive for specific product (a fragment of the *Chlamydia trachomatis* genome). The samples free of *Chlamydia trachomatis* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification DNA sample in the presence of *Chlamydia pneumonia, Chlamydia psittaci, Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum, Neisseria gonorrhoeae, Candida albicans, Streptococcus sp., Staphylococcus sp*., as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. In a determination of analytical **sensitivity** the **Chlamydia trachomatis REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Chlamydia trachomatis* 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
10	94	94	100
5	94	94	100
2	94	82	87
0	94	0	0

Sensitivity of *Chlamydia tracho*matis DNA in the sample depends on the sampling and the final volume of the extracted DNA (elution volume).

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Chlamydia trachomatis* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID** (at elution in 300 µL)	**PREP-RAPID**
– scraping of epithelial cells in 500 µL transport medium; – ejaculate in 500 µL transport medium; – prostate fluid in 500 µL of transport medium; – urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 488;

Diagnostic sensitivity (95% CI) - 98.5% (94.1-98.5%);

Diagnostic specificity (95% CI) – 100% (99.3-100%).

NOTE - The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** REF P-001/1EU, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU 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 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 official representative in EU by quality issues of **Chlamydia trachomatis REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
	Do not reuse	NON STERILE	Non-sterile
EC REP	Authorized representative in the European Community	CONTROL +	Positive control

REF	R1-P101-S3/9EU R1-P101-23/9EU R1-P101-UA/9EU	VER	560-3.2024.04.22

283-4 2024-04-22

For professional use only

Mycoplasma genitalium REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF R1-P103-S3/9EU
R1-P103-23/9EU

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1. INTENDED USE

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is designed to detect *Mycoplasma genitalium* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, control of the treatment of infection caused by *Mycoplasma genitalium*.

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

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Mycoplasma genitalium* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Mycoplasma genitalium	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Mycoplasma genitalium 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 **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P103-S3/9EU and R1-P103-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P103-S3/9EU

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

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P103-S3/9EU);
- 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 20 µL, 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Mycoplasma genitalium REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures from 2°C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Mycoplasma genitalium 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 **Mycoplasma genitalium REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Mycoplasma genitalium REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

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. 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.

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 **Mycoplasma genitalium REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the urogenital tract, urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

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

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

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

Assay procedure for package S

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

When using package S (R1-P103-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Mycoplasma genitalium REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the Mycoplasma genitalium 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.

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

For **Mycoplasma genitalium 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.

For **Mycoplasma genitalium 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Mycoplasma genitalium* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Mycoplasma genitalium* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case,

amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical **specificity** of the **Mycoplasma genitalium REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Mycoplasma genitalium* DNA are to be registered positive for specific product (a fragment of the *Mycoplasma genitalium* genome). The samples free of *Mycoplasma genitalium* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Trichomonas vaginalis, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Mycoplasma genitalium REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Mycoplasma genitalium* 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
10	94	94	100
5	94	94	100
2	94	86	91.5
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Mycoplasma genitalium* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 398;

Diagnostic sensitivity (95% CI) - 98.0% (92.0-98.0%);

Diagnostic specificity (95% CI) – 100% (99.1-100%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Mycoplasma genitalium REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

DNA-TECHNOLOGY

285-4 2024-04-22

CE IVD

For professional use only

Ureaplasma urealyticum REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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R1-P106-23/9EU

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1. INTENDED USE

The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is designed to detect *Ureaplasma urealyticum* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the urethra, cervix, or posterolateral vaginal wall, urine, prostate fluid, ejaculate.

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

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

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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Ureaplasma urealyticum* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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 urealyticum	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Ureaplasma urealyticum 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 urealyticum REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P106-S3/9EU and R1-P106-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P106-S3/9EU

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

The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-GS PLUS** (REF P-003/2EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P106-S3/9EU);
- 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 20 µL, 50 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Ureaplasma urealyticum 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 **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Ureaplasma urealyticum REAL-TIME PCR Detection Kit**.

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 **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the genitourinary tract (urethra, cervix or posterolateral vaginal wall), urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by “DNA-Technology Research&Production”, LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-GS PLUS, PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS** and **PREP-GS PLUS** leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

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

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS, PREP-GS PLUS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

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

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

Assay procedure for package S

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

When using package S (R1-P106-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

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

In case of using **PREP-GS, PREP-GS PLUS** extraction kits. 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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-“ tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Ureaplasma urealyticum* 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.

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

For **Ureaplasma urealyticum 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.

For **Ureaplasma urealyticum 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Ureaplasma urealyticum* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Ureaplasma urealyticum* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Ureaplasma urealyticum* DNA are to be registered positive for specific product (a fragment of the *Ureaplasma urealyticum* genome). The samples free of *Ureaplasma urealyticum* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Mycoplasma genitalium, Mycoplasma hominis, Gardnerella vaginalis, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Trichomonas vaginalis, Ureaplasma parvum, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Ureaplasma urealyticum REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 10 copies of *Ureaplasma urealyticum* 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	86	91.5
2	94	76	80.8
0	94	0	0

Sensitivity of *Ureaplasma urealyticum* 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 urealyticum* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits				
	PREP-NA	**PREP-GS**	**PREP-GS PLUS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	100 copies /sample	200 copies /sample	600 copies /sample	600 copies /sample	1000 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 298;

Diagnostic sensitivity (95% CI) - 100% (95.4-100%);

Diagnostic specificity (95% CI) – 99.6% (98.2-99.6%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-GS PLUS** (REF P-003/2EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Ureaplasma urealyticum REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

DNA-TECHNOLOGY

287-4 2024-04-22

IVD

For professional use only

Gardnerella vaginalis REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is designed to detect *Gardnerella vaginalis* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, control of the treatment of infection caused by *Gardnerella vaginalis*.

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

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Gardnerella vaginalis* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Gardnerella vaginalis	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Gardnerella vaginalis 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 **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P108-S3/9EU and R1-P108-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P108-S3/9EU

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

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P108-S3/9EU);
- 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 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Gardnerella vaginalis REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures from 2°C to 8°C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Gardnerella vaginalis 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 **Gardnerella vaginalis REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Gardnerella vaginalis REAL-TIME PCR Detection Kit**.

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 **Gardnerella vaginalis REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by

a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

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

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 in volumes as indicated.

Assay procedure for package S

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

When using package S (R1-P108-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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	35		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]			Holding		Holding
[1] – holding at 25°C is allowed						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	35				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	35				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Gardnerella vaginalis REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Gardnerella vaginalis* 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.

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 **Gardnerella vaginalis 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.

For **Gardnerella vaginalis 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Gardnerella vaginalis* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Gardnerella vaginalis* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case,

amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Gardnerella vaginalis REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Gardnerella vaginalis* DNA are to be registered positive for specific product (a fragment of the *Gardnerella vaginalis* genome). The samples free of *Gardnerella vaginalis* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Streptococcus sp., Staphylococcus sp.,* as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Gardnerella vaginalis REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 50 copies of *Gardnerella vaginalis* 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
100	94	94	100
50	94	94	100
25	94	19	20.2
0	94	0	0

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

Sensitivity of 50 copies per amplification tube corresponds to the following values of the DNA concentration of *Gardnerella vaginalis* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	500 copies /sample	1000 copies /sample	3000 copies /sample	5000 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 398;

Diagnostic sensitivity (95% CI) - 98.9% (97.2-99.6%);

Diagnostic specificity (95% CI) – 97% (93.9-98.6%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Gardnerella vaginalis REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

DNA-TECHNOLOGY

235-5 2024-04-22

CE IVD

For professional use only

Mycoplasma hominis REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Mycoplasma hominis REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Mycoplasma hominis REAL-TIME PCR Detection Kit** is designed to detect *Mycoplasma hominis* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, control of the treatment of infection caused by *Mycoplasma hominis*.

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

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Mycoplasma hominis* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Mycoplasma hominis	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Mycoplasma hominis 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 **Mycoplasma hominis REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Mycoplasma hominis REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P102-S3/9EU and R1-P102-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P102-S3/9EU

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

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: sterile single use swabs, cytobrushes, cotton swabs e.t.c for sampling of biomaterial;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample;
- For prostate fluid, ejaculate, urine: sterile containers with a volume of up to 60 mL.

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 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS**

(REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for 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 20 µL, 50 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Mycoplasma hominis REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures corresponding to storage conditions of the kit components over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Mycoplasma hominis 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 **Mycoplasma hominis REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Mycoplasma hominis REAL-TIME PCR Detection Kit**.

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 **Mycoplasma hominis REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by

a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

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

Assay procedure for package S

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

When using package S (R1-P102-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction kit.** The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Mycoplasma hominis REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the Mycoplasma hominis 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.

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

For **Mycoplasma hominis 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.

For **Mycoplasma hominis 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Mycoplasma hominis* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Mycoplasma hominis* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors,

incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Mycoplasma hominis REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Mycoplasma hominis* DNA are to be registered positive for specific product (a fragment of the *Mycoplasma hominis* genome). The samples free of *Mycoplasma hominis* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma genitalium, Ureaplasma parvum, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Trihomonas vaginalis, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Mycoplasma hominis REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Mycoplasma hominis* 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
10	94	94	100
5	94	94	100
2	94	85	90.4
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Mycoplasma hominis* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 398;

Diagnostic sensitivity (95% CI) - 97.5% (92.5-99.5%);

Diagnostic specificity (95% CI) – 99.1% (97.8-99.6%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Mycoplasma hominis REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
NON STERILE	Non-sterile	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Do not reuse

REF	R1-P102-S3/9EU R1-P102-23/9EU	VER	235-5.2024.04.22

DNA-TECHNOLOGY

286-5 2024-04-22

CE IVD

For professional use only

Trichomonas vaginalis REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF R1-P107-S3/9EU
R1-P107-23/9EU

VER 286-5.2024.04.22

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1. INTENDED USE

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is designed to detect *Trichomonas vaginalis* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, control of the treatment of infection caused by *Trichomonas vaginalis*.

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

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Trichomonas vaginalis* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Trichomonas vaginalis	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Trichomonas vaginalis 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 **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P107-S3/9EU and R1-P107-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P107-S3/9EU

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

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P107-S3/9EU);
- 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 20 µL, 50 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Trichomonas vaginalis REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures corresponding to storage conditions of the kit components over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Trichomonas vaginalis 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 **Trichomonas vaginalis REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Trichomonas vaginalis REAL-TIME PCR Detection Kit**.

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 **Trichomonas vaginalis REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by

a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

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 in volumes as indicated.

Assay procedure for package S

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

When using package S (R1-P107-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Trichomonas vaginalis REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Trichomonas vaginalis* 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.

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

For **Trichomonas vaginalis 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.

For **Trichomonas vaginalis 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Trichomonas vaginalis* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Trichomonas vaginalis* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Trichomonas vaginalis REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Trichomonas vaginalis* DNA are to be registered positive for specific product (a fragment of the *Trichomonas vaginalis* genome). The samples free of *Trichomonas vaginalis* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum, Neisseria gonorrhoeae, Candida albicans, Chlamydia trachomatis, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Trichomonas vaginalis REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Trichomonas vaginalis* 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
10	94	94	100
5	94	94	100
2	94	73	77.6
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Trichomonas vaginalis* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 398;

Diagnostic sensitivity (95% CI) - 100% (76.8-100%);

Diagnostic specificity (95% CI) – 99.7% (99.0-99.7%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Trichomonas vaginalis REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

DNA-TECHNOLOGY

288-5 2024-04-22

CE IVD

For professional use only

Neisseria gonorrhoeae REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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R1-P109-23/9EU

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1. INTENDED USE

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is designed to detect *Neisseria gonorrhoeae* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, control of the treatment of infection caused by *Neisseria gonorrhoeae*.

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

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Neisseria gonorrhoeae* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Neisseria gonorrhoeae	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Neisseria gonorrhoeae 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 **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P109-S3/9EU and R1-P109-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P109-S3/9EU

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

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P109-S3/9EU);
- 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 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures corresponding to storage conditions of the kit components over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Neisseria gonorrhoeae 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 **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit**.

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 **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs from the genitourinary tract, urine, prostate fluid, ejaculate, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra)

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

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

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by “DNA-Technology Research&Production”, LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 ° C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB-RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by

a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

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

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 in volumes as indicated.

Assay procedure for package S

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

When using package S (R1-P109-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

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

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Neisseria gonorrhoeae* 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.

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 **Neisseria gonorrhoeae 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.

For **Neisseria gonorrhoeae 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Neisseria gonorrhoeae* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Neisseria gonorrhoeae* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case,

amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Neisseria gonorrhoeae* DNA are to be registered positive for specific product (a fragment of the *Neisseria gonorrhoeae* genome). The samples free of *Neisseria gonorrhoeae* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum, Trichomonas vaginalis, Candida albicans, Chlamydia trachomatis, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Neisseria gonorrhoeae* 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
10	94	94	100
5	94	94	100
2	94	83	88.3
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Neisseria gonorrhoeae* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 398;

Diagnostic sensitivity (95% CI) - 100% (88.2-100%);

Diagnostic specificity (95% CI) – 99.7% (98.9-99.7%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

289-4 2024-04-22

For professional use only

Candida albicans REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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R1-P110-23/9EU

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1. INTENDED USE

The **Candida albicans REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Candida albicans REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Candida albicans REAL-TIME PCR Detection Kit** is designed to detect *Candida albicans* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs (from the genitourinary tract, oropharynx, rectum, conjunctiva of the eye), urine, prostate fluid, ejaculate.

Indications for the use: symptoms of infectious or inflammatory diseases of the genitourinary tract, rectum, oropharynx, eyes, control of the treatment of infection caused by *Candida albicans*.

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

The **Candida albicans REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **Candida albicans REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Candida albicans* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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)
Candida albicans	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Candida albicans 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 **Candida albicans REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Candida albicans REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Candida albicans REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P110-S3/9EU and R1-P110-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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		

*- for detection kit packaged in strips REF R1-P110-S3/9EU

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

The **Candida albicans REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control).

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** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;

- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P110-S3/9EU);
- 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 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Candida albicans REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over 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 can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Candida albicans 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 **Candida albicans REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Candida albicans REAL-TIME PCR Detection Kit**.

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 **Candida albicans REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial cell swabs (from the urogenital tract, rectum, oropharynx, conjunctiva of the eyes), prostate fluid, ejaculate, urine, depending on professional prescription.

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.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

Genitourinary swabs sampling (cervical canal, vagina, urethra), rectum sampling

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

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

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by "DNA-Technology Research&Production", LLC).

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

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

Samples may be stored in physiological saline at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1. The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25 °C.

Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2. Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by

a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the 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 the prostate fluid is hermetically screwed and marked.

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

Residual urine after prostate massage

Before residual urine after prostate massage, sexual abstinence is recommended for 3 days before the examination.

The patient urinates in the toilet, leaving part of the urine in the bladder.

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

The prostate massage is carried out for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: with a soft prostate - slight pressure is carried out, with a dense consistency of the prostate - the pressure force is increased.

After the end of the massage, the first 10-15 mL of the urine is collected in a sterile container with a volume of up to 60 mL.

Container is tightly screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month.

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

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

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

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 in volumes as indicated.

Assay procedure for package S

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

When using package S (R1-P110-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 Mark 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

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 in 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 in a vortex mixer.

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.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

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

8.10 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 (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

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

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Candida albicans REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Candida albicans* 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.

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 **Candida albicans 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.

For **Candida albicans 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Candida albicans* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Candida albicans* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical **specificity** of the **Candida albicans REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Candida albicans* DNA are to be registered positive for specific product (a fragment of the *Candida albicans* genome). The samples free of *Candida albicans* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Chlamydia trachomatis, Ureaplasma parvum, Neisseria gonorrhoeae, Candida glabrata, Candida tropicalis, Streptococcus sp., Staphylococcus sp.*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical **sensitivity**, the **Candida albicans REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Candida albicans* 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
10	94	94	100
5	94	94	100
2	94	42	44.6
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Candida albicans* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution in 300 µL)**	**PREP-RAPID**
- epithelial cell swabs in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 491;

Diagnostic sensitivity (95% CI) – 98.3% (96.0-99.2%);

Diagnostic specificity (95% CI) – 99.4% (98.1-99.9%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** (REF P-001/1EU), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, 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 issueswith 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 official representative in EU by quality issues of **Candida albicans REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	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	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution

769-2 2024-09-03

For professional use only

HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit

INSTRUCTION FOR USE

EC REP

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REF R1-P325-S3/9EU
R1-P325-23/9EU

VER 769-2.2024.09.03

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1. INTENDED USE

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is intended for research and diagnostic applications. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed to detect HPV nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina.

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed for qualitative DNA detection of fourteen types of human papillomaviruses with high carcinogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 types) associated with cervical cancer, and differential detection of HPV 16, 18 and 45 types in human biological material.
Indications for the use: screening for precancer and cervical cancer, symptoms of HPV infection.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit.**

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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 detecting of the results in real time; qualitative multiplex 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 the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by paraffin layer. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter fluorescent dyes and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

Amplification mixture includes a system for amplification of a fragment of human genomic DNA (sample intake control, SIC), which allows to evaluate the presence of DNA in the amplification tube and control all stages of the analysis.

DNA probes used to detect specific DNA amplification products include the fluorescent tags Fam, Rox, Cy5, Cy5.5. The DNA probe used to detect the amplification product of the sample intake control includes the fluorescent dye Hex.

The application of several fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
16	SIC	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	18	45

The automatic analysis is available on "DNA-Technology" made instruments: DTlite[1] or DTprime[2] REAL-TIME Thermal Cyclers for **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2-3.

Table 2. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** content, package S (standard), strips, for R1-P325-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white 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		

* - marking as C+ is allowed

Table 3. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** content, package S (standard), tubes R1-P325-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white 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

* - marking as C+ is allowed

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

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is intended for single use and designed for 96 tests (no more than 94 defined samples, one positive control and one negative control).

[1] - supported by 5S1, 5S2 instruments.
[2] - supported by 5M1, 5M3, 5M6 instruments.
If using detecting thermal cyclers with 4 detection channels, papilloma type 45 is not detected.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, cytobrushes, cotton swabs e.t.c for sampling of biomaterial;
- Sterile tubes containing transport medium: “DNA-Technology” made **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-65 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 1.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 20 µL, 200 µL, 1000 µL);
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-NA** REF P-002/1EU**, PREP-GS** REF P-003/1EU**, PREP-RAPID** REF P-001/1EU (not applicable to male urethral swabs) and **PREP-MB RAPID** REF P-116-A/8EU, REF P-116-N/4EU extraction kits are recommended)**;**
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- 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);
- 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** must be stored at temperatures from 2 °C to 8 °C over 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.

It is allowed to transport the kit in thermobox 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.

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

– components of the kit should be stored at temperatures from 2 °C to 8 °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 under undue regime should not be used.

An expired the **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed to detect DNA extracted from the epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina.

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 maximum concentrations of interfering substances at which no PCR inhibition was observed are shown in the table below:

Type of biomaterial	**Interfering substance**	**Studied concentration in the sample**
Endogenous substances		
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Hemoglobin*	0.35 mg/mL
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Mucus (mucin)	20 %
Exogenous substances		
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Isopropyl alcohol*	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Methyl acetate*	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Chlorhexidine bigluconate	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Miramistin®	10%

*** -** interfering substance may be present in DNA preparation due to its incomplete removement in the course of DNA extraction.

General requirements

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

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.

Professional prescription is required to localize the place of sampling. The decision must be based on a patient's complaints and clinical signs, and made by the physician in charge.

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

Sample collection

Epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina

Sample taking is made with special sterile single-use tools – probes, cytobrushes, 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.

It is allowed to use swabs to take the material on your own.

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

The taking of the swabs is carried out in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.

Order of taking:

1 Open the tube.

2 Take biological material with a sterile swab.

3 Put the swab into the tube with transport medium and rinse it thoroughly for 10-15 seconds. Avoid spraying of solution.

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

5 Close the tube tightly and mark it.

If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material with a new probe into a new tube.

Before sampling procedure, it is necessary to remove the mucus with a cotton tampon.

The features of the vaginal sampling using a device for self-sampling

The sampling is carried out in accordance with the instructions for use of the device.

Material is taken into the transport & fixation medium for liquid cytology in accordance with the Instruction for the transport & fixation medium for liquid cytology.

Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

Transportation and storage of the samples

Transport and storage conditions for epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina are determined by the instructions for the transport media used for sample transport and storage or the instructions for the recommended DNA extraction kits.

Sample preparation

Epithelial smears/samples from the mucous membrane of the cervical canal and the vagina, collected in transport media for PCR studies.

1 Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperature (from 18 °C to 25 °C).

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.

The resulting material is ready for DNA extraction.

Vaginal material taken using a self-sampling device

1 Add 500 µL of physiological saline solution to the tube (or other container specified in the instructions for the self-sampling device ("tube") that holds the tip of the device.

2 Shake the tube for 15 seconds.

3 Remove the tip of the device from the tube and discard it.

4 Transfer to a clean 1.5 mL tube.

5 Centrifuge the tube at RCF(g) 16000 for 10 minutes.

6 Remove the supernatant, leaving approximately 100 µL in the tube (precipitate+liquid fraction).

The resulting material is ready for DNA extraction.

Scrapes taken in transport & fixation medium for liquid cytology

When fixing in some alcoholic transport media for liquid cytology, for example, in the preservative liquid BD SurePath (Becton Dickinson, USA), cross-linking of nucleic acids with proteins occurs, so pre-treatment of samples in order to release DNA from protein-associated complexes and cell lysis is necessary.

Scrapes taken in the transport & fixation medium for liquid cytology BD SurePath (Becton Dickinson, USA) must be pretreated using the **PREP-PK** reagent kit ("DNA-Technology", LLC) in accordance with the instructions to the **PREP-PK** reagent kit.

Transport & fixation media PreservCyt®ThinPrep (Hologic Inc, USA) or CellPrep (Biodyne, Korea) do not require sample pretreatment using the **PREP-PK** reagent kit.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS, PREP-RAPID** 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 from an extraction kit can be used as a negative control in volumes as indicated.

Assay procedure

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

ATTENTION! When using package S (R1-P325-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 Mark tubes with 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

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

8.5 Vortex the tubes with samples, “C-” and “C+” for 3-5 seconds, then 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 in a vortex mixer.

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

ATTENTION! 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 before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.6 Add 5 µL of DNA sample into corresponding tubes. Do not add DNA into the "C-" and "C+" tubes. Avoid paraffin layer break.

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

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

8.9 Spin tubes/strips for 3-5 seconds.

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

8.11 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/strips in the thermal unit (see 8.10) and run PCR. See Table 4.

Table 4. 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						

9. CONTROLS

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** contains positive control sample. Positive control is a cloned part of the HPV genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix contains sample intake control (SIC). Sample intake control (SIC) estimates the amount of human DNA in the tube. 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 from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

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

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 PCR results is held in automatic mode. Analysis will be performed by Real-Time PCR application. The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube. Operator can create, save and print a report.

When the PCR is complete, the program displays a "+" or "-" in the table in the "Result" column. In this case a conclusion on the results of the study is given.

Interpretation of the PCR results should be performed according to the Table 5.

Table 5. Interpretation of the PCR results

Detection channel					**Interpretation**
Fam	**Hex**	**Rox**	**Cy5**	**Cy5.5**	
+	Is not considered	+	-	-	DNA HPV16 is detected
-	Is not considered	+	+	-	DNA HPV18 is detected
-	Is not considered	+	-	+	DNA HPV45 is detected
-	Is not considered	+	-	-	DNA HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68 is detected (no differentiation)
-	+	-	-	-	DNA HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 is not detected
-	-	-	-	-	Invalid result*
Positive control sample					
+	+	+	+	+	The results are valid
Negative control sample					
-	-	-	-	-	The results are valid

* - Invalid results can be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect performance of the analysis protocol; failure to follow the amplification temperature regime, etc. In this case, it is necessary to repeat PCR with the existing DNA preparation, or repeat DNA extraction and PCR, or repeat collection of biological material (performed sequentially).
In case of results for negative control sample different from those in Table 5, the results of all series are considered invalid. In this case decontamination procedures are required. In this case special measures to detect and eliminate possible contamination are required.
In case of results for positive control sample different from those in Table 5, it is required to repeat amplification for all series.

11. SPECIFICATIONS

a. The analytical **specificity** of the **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

In samples of human biological material containing human papillomavirus type 16 DNA, the detection thermocycler software records positive amplification results for the specific product on the Fam and Rox channels during amplification.

In samples of human biological material containing human papillomavirus type 18 DNA, detection thermocycler software records positive amplification results for the specific product on the Cy5 and Rox channels during amplification.

In samples of human biological material containing human papillomavirus type 45 DNA, detection thermocycler software detects positive amplification results for the specific product on the Cy5.5 and Rox channels during amplification.

In samples of human biological material that contain DNA from one or more human papillomavirus (HPV) types at high carcinogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), the detection thermocycler software records positive amplification results for the specific product on the Rox channel during amplification.

In samples of biological material that do not contain DNA of 14 types of highly carcinogenic human papillomaviruses (HPV) (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), the detection thermocycler software detects negative amplification results for the specific product on the Fam, Rox, Cy5, and Cy5.5 detection channels and positive amplification results for SIC on the Hex detection channel.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum*, *Ureaplasma parvum, Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Candida albicans*, *Streptococcus sp*., *Staphylococcus sp*., *Lactobacillus spp*., EBV, HHV6, HHV8, HSV1, HSV2, VZV.

b. Analytical **sensitivity**

Analytical sensitivity is 5 DNA copies of each type of human papillomavirus per amplification tube, which corresponds to 10^3 DNA copies of each type of human papillomavirus per 1.0 mL.

c. Diagnostic characteristics

Number of samples (n) - 436

Analyte detected	Diagnostic sensitivity (95% CI)	Diagnostic specificity (95% CI)
HPV16	100.00% (96.38%-100.00%)	100.00% (98.91%-100.00%)
HPV18	100.00% (83.16%-100.00%)	100.00% (99.12%-100.00%)
HPV45	100.00% (69.15%-100.00%)	100.00% (99.14%-100.00%)
HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68 (no differentiation) (excluding types 16, 18 and 45)	100.00% (98.54%-100.00%)	100.00% (98.04%-100.00%)

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU**, PREP-RAPID** REF P-001/1EU and **PREP-MB RAPID** REF P-116-A/8EU, REF P-116-N/4EU extraction kits.

12. TROUBLESHOOTING

Table 6. 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
SIC	-	PCR inhibition Insufficient amount of DNA	Repeat whole test Resample

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

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the abovementioned 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 official representative in EU by quality issues of **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit.**

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

REF	R1-P325-S3/9EU R1-P325-23/9EU	VER	769-2.2024.09.03

For professional use only

CMV REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF

R1-P204-S3/9EU

R1-P204-23/9EU

R1-P204-UA/9EU

VER

549-3.2024.04.22

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1. INTENDED USE

The **CMV REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **CMV REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) for qualitative pathogen detection. The **CMV REAL-TIME PCR Detection Kit** is designed to detect CMV nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: saliva, urine, prostate fluid, ejaculate, swabs from urethra and conjunctiva of the eye, cervix, or posterolateral vaginal wall, breast milk, peripheral blood mononuclear cells, liquor, amniotic fluid, tissue samples.

CMV infection is typically unnoticed in healthy people, but can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or newborn infants. After infection, CMV remains latent within the body throughout life and can be reactivated at any time. Eventually, it may cause mucoepidermoid carcinoma and possibly other malignancies such as prostate cancer.

Congenital CMV is the leading infectious cause of deafness, learning disabilities, and mental retardation in children. CMV also seems to have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality.

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

The **CMV REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The **CMV REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the CMV product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of two fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. 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
CMV	IC	-	-	-

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers (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 **CMV REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) real-time thermal cyclers.

3. CONTENT

The detailed description of content is represented in the Tables 2 - 3.

Table 2. The **CMV REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P204-23/9EU and R1-P204-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 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[1]	12 8-caps		

Table 3. The **CMV REAL-TIME PCR Detection Kit** content, package U (universal) for R1-P204-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or 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 (no more than 94 defined samples, one positive control and one negative control) for package S.

Package U is designed to carry out 96 tests if at least 5 samples in one study are amplified (3 unknown samples, positive and negative control samples).

[1] - for detection kit packaged in strips REF R1-P204-S3/9EU

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

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

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Physiological saline solution 0.9% NaCl (Sterile);
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-RAPID** REF P-001/1EU (not applicable to male urethral swabs), **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits are recommended);
- High speed centrifuge (RCF(g) not less than 16000);
- Solid-state thermostat (temperature range 50-98°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 50 µL, 200 µL, 1000 µL);
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips.

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 **CMV REAL-TIME PCR Detection Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °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 TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport TechnoTaq MAX polymerase at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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;
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

An expired **CMV 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 **CMV REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **CMV REAL-TIME PCR Detection Kit**.

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. 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, reverse transcription, 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.

Do not open the tubes after amplification. 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. 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 instruction to use.

7. SAMPLES

The **CMV REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from saliva, urine, prostate fluid, ejaculate, swabs from urethra and conjunctiva of the eye, cervix, or posterolateral vaginal wall, breast milk, peripheral blood mononuclear cells, liquor, amniotic fluid, tissue samples, depending on professional prescription.

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 amplification.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

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.

Following medicines have no effect on the amplification of the laboratory control sample and internal control: chlorhexidine bigluconate – 5%, Miramistin ® - 5%.

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

The features of genitourinary swabs sampling:

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

The features of the posterior vaginal vault sampling:

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

The features of the urethral sampling:

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

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

In the presence of purulent discharge, the 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.

The features of the cervical sampling:

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

The features of the eye conjunctiva sampling:

If the abundant purulent discharge is presence, it is removed with a sterile cotton tampon moistened with saline. Sampling is taken from the inner surface of the lower eyelid by the movement to the inner corner of the eye slit. It is necessary to hold the eyelid with hands so that the eyelashes do not touch the probe.

Genitourinary swabs sampling (cervical canal, vagina, urethra) and eye conjunctiva sampling

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

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

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

The taking of the scrapes is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a 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** (manufactured by “DNA-Technology Research&Production”, LLC).

ATTENTION! PREP-RAPID is not recommended for DNA extraction from male urogenital scrapes.

Order of taking:

1. Open the tube.

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

3. Tightly close the tube, mark the tube.

ATTENTION! Samples may be stored at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

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

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1 The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25°C.

NOTE - Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2 Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

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

The first portion of morning urine

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

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

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

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

The prostate fluid

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

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

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

After the end of the massage, the released prostate fluid in the form of a free flowing drop (0.15-1.0 mL) is collected in a 2 mL single dry sterile tube or a container with a volume of up to 60 mL.

The container with the prostate fluid is hermetically screwed and marked.

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

Residual urine after prostate massage

Before residual urine after prostate massage, sexual abstinence is recommended for 3 days before the examination.

The patient urinates in the toilet, leaving part of the urine in the bladder.

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

The prostate massage is carried out for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: with a soft prostate - slight pressure is carried out, with a dense consistency of the prostate - the pressure force is increased.

After the end of the massage, the first 10-15 mL of the urine is collected in a sterile container with a volume of up to 60 mL.

Container is tightly screwed and marked.

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

Ejaculate

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

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

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

The container with ejaculate is hermetically closed and marked.

Saliva, liquor, amniotic fluid

1. Saliva, liquor, amniotic fluid (about 500 µL) is collected in a sterile container and tightly closed.
2. Then 500 µL of the material is transferred into a 1.5 mL tube.
3. The tube is centrifuged at RCF(g) 13000 for 10 minutes at room temperatures from 18 °C to 25 °C.
4. The supernatant is removed, leaving 50 µL in tube (precipitate + liquid fraction).
5. 500 µL of a sterile saline solution is added to the precipitate.
6. The tube is centrifuged at RCF(g) 13000 for 10 minutes at room temperatures from 18 °C to 25 °C.
7. The supernatant is removed, leaving 100 µL in tube (precipitate + liquid fraction).

Breast milk

Material is collected into a sterile container and tightly closed.

The material is gently shaked and 1.0 mL of the material is transferred into a 1.5 mL tube.

Note. The milk collection period is not more than 24 hours. Storage for the entire collection period at temperatures from 2 °C to 8 °C.

Peripheral/umbilical cord blood

Peripheral/cord blood sampling is carried out in vacuum tubes. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example disodium salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 2 – 3 times.

Tissue samples

Pieces of the tissue (diameter is not more than 5 mm) are placed in sterile 2 mL tubes with appropriate transport medium. The test tube is tightly closed and marked.

Transportation and storage of the samples

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 18 °C to minus 22 °C for one month

NOTE - The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID, PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kit's instruction for use.

8. PROCEDURE

DNA extracting from biological material.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from men urogenital swabs.

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

8.1 Assay procedure for package S:

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

ATTENTION! When using package S, strips, strictly observe the completeness of the strips and caps for them. Do not use caps for strips from other kits!

8.1.1 Mark tubes with 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 Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.1.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close tubes.

8.1.5 Vortex the tubes with samples, "C-" and "C+" for 3-5 seconds, then 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 in a vortex mixer.

ATTENTION! In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in 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-" and "C+" tubes. Avoid paraffin layer break.

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube and positive control (C+) into corresponding tube. Avoid paraffin layer break. Spin the tubes for 3-5 seconds.

8.1.8 Spin tubes/strips for 3-5 seconds.

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

8.1.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], 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 Tables 4, 8.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 5-8.

8.2 Assay procedure for package U:

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

Example: to test 4 samples in one PCR run, 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 and spin down drops for 1-3 seconds.

8.2.3 Add 6.0 µL of PCR-mix into the each marked tube for samples to be tested.

8.2.4 Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin for down drops for 1-3 seconds.

ATTENTION! TechnoTaq MAX polymerase must be stored at temperatures from minus 18°C to minus 22°C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase.

Add into one tube:

- 6,0×(N+1) µL of PCR-buffer,
- 0,3×(N+1) µL of TechnoTaq MAX polymerase,

N — number of the marked tubes including "C-" and "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 TechnoTaq MAX 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 prepared mixture for 3-5 seconds, then spin down drops for 1-3 seconds.

ATTENTION! The mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared just prior to use.

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

ATTENTION! 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 amplification mix.

8.2.8 Vortex the tubes with sample, "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 in a vortex mixer.

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

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

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

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

8.2.9 Add 6.0 µL of DNA sample into corresponding PCR-tubes. Do not add DNA into the "C-" and "C+" tubes. Avoid paraffin layer break.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube and positive control (C+) into corresponding tube. Avoid paraffin layer break. Close the tubes tightly.

8.2.11 Spin tubes for 3-5 seconds.

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

8.2.13 Launch the operating software for DT instrument[4]. Add corresponding test[5], 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 Tables 8-12.

8.3 Assay procedure for package U using DTstream:

8.3.1. Vortex the tube with PCR-mix for 3-5 seconds and spin down drops for 1-3 seconds.

8.3.2. Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! TechnoTaq MAX polymerase must be stored at temperatures from minus 18°C to minus 22°C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.3.3. Following the DTstream software instructions, prepare a mixture of PCR-buffer with TechnoTaq MAX polymerase in a separate test tube.

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

8.3.5. 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 in a vortex mixer.

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 in a vortex mixer.

8.3.6. Set tubes with PCR-mix, PCR-buffer and TechnoTaq MAX polymerase mixture, DNA sample, positive control and negative control and microplate for PCR to the DTstream and dispense the components according to the instruction manual.

[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.

8.3.7. After completion of the program on the DTstream, set gently, without shaking, the microplate to the DTpack.

8.3.8. Carry out the procedure of sealing the microplate by thermal film in accordance with the instructions to the DTpack.

8.3.9. Spin the microplate at RCF(g) 1000 for 30 seconds.

8.3.10. Set the microplate into the Real-time Thermal Cycler.

8.3.11. Launch the operating software for DT instrument[6]. Add corresponding test[7], 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 Tables 8-12.

Table 4. 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						

Table 5. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

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

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

Table 6. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 7. The PCR program for Rotor-Gene thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	

* Take the measurement.

Table 8. Detection channels

Fam (Green)	Hex (Yellow)	Rox	Cy5	Cy5.5
Specific product and C+	IC	-	-	-

Table 9. 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	5	15		Cycle
	94	0	5			
2	94	5	0	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		v	
4	94	0	10	45		Cycle
	64	0	15		v	
5	94	0	5	1		Cycle
6	10[1]	…	…	Holding		Holding
[1] – holding at 25°C is allowed						

Table 10. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	5 min	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 11. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	**Repeats**	**Step**	**Dwell time**	**Setpoint, ºC**	**PCR/Melt Data Acquisition**
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	5 min	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 12. The PCR program for Rotor-Gene thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	

9. CONTROLS

The **CMV REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the CMV 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.

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

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.

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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers the analysis performed automatically. In other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing CMV DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of CMV DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current instruction for use). The cutoff Ct values for Rotor-Gene thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical **specificity** of the **CMV REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with CMV DNA are to be registered positive for specific product (a fragment of the CMV genome). The samples free of CMV DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification DNA sample in the presence of Herpes simplex virus 1, 2, Human herpesvirus 6, 8, HPV 6, 11, Epstein Barr virus, Varicella zoster virus, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, *Neisseria gonorrhoeae*, *Candida albicans*, *Streptococcus sp*., *Staphylococcus sp*., as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. In a determination of analytical **sensitivity** the **CMV REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of CMV 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
10	94	94	100
5	94	93	98,9
2	94	73	77.7
1	94	56	59.6
0	94	0	0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of CMV in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	PREP-GS	PREP-MB RAPID (at elution in 300 µL)	PREP-RAPID
- scraping of epithelial cells in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 424;

Diagnostic sensitivity (95% CI) - 98.0% (91.8-98.0%);

Diagnostic specificity (95% CI) – 100% (99.2-100%).

NOTE - The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** REF P-001/1EU, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU kits.

12. TROUBLESHOOTING

Table 13. 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

“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.

Contact our customer service with quality issues of **CMV REAL-TIME PCR Detection Kit**:

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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14. KEY TO SYMBOLS

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
VER	Version	CONTROL +	Positive control
	Do not use reuse		Caution
EC REP	Authorized representative in the European Community		

REF

R1-P204-S3/9EU

R1-P204-23/9EU

R1-P204-UA/9EU

VER

549-3.2024.04.22

ДНК-ТЕХНОЛОГИЯ

1093 2025-02-15

IVD

ИНСТРУКЦИЯ

по применению набора реагентов

для выявления ДНК Herpes Simplex Virus 1, Herpes Simplex Virus 2, Cytomegalovirus

методом ПЦР в режиме реального времени

HSV1/HSV2/CMV ГерпесКомплекс

Регистрационное удостоверение

№ РЗН 2025/24371 от 07 февраля 2025 года

ВНИМАНИЕ! Изучите инструкцию перед началом работы

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СПИСОК СОКРАЩЕНИЙ И ОБОЗНАЧЕНИЙ

В настоящей инструкции используются следующие сокращения и обозначения:

CMV	- от англ. Cytomegalovirus
HSV1	- от англ. Herpes simplex virus 1
HSV2	- от англ. Herpes simplex virus 2
RCF	- от англ. relative centrifugal force, относительное ускорение центрифуги
ВК	- внутренний контроль
ДНК	- дезоксирибонуклеиновая кислота
ДНКазы	- дезоксирибонуклеазы
К-	- отрицательный контрольный образец
К+	- положительный контрольный образец
ЛКО	- лабораторный контрольный образец
НК	- нуклеиновые кислоты (РНК и ДНК)
ПЦР	- полимеразная цепная реакция
РНКазы	- рибонуклеазы

1 ПРЕДНАЗНАЧЕННОЕ ПРИМЕНЕНИЕ

1.1 Полное наименование набора реагентов: Набор реагентов для выявления ДНК Herpes Simplex Virus 1, Herpes Simplex Virus 2, Cytomegalovirus методом ПЦР в режиме реального времени (HSV1/HSV2/CMV ГерпесКомплекс), далее по тексту – набор реагентов.

1.2 Назначение: набор реагентов предназначен для выявления ДНК Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus в биологическом материале человека (моча, соскобы эпителиальных клеток из урогенитального тракта) методом ПЦР в режиме реального времени.

1.3 Функциональное назначение: диагностика *in vitro*.

1.4 Показания к проведению анализа: симптомы инфекционного заболевания урогенитального тракта.

Противопоказаний к применению нет.

1.5 Популяционные и демографические аспекты: применение набора реагентов не зависит от популяционных и демографических аспектов.

1.6 Область применения: набор реагентов может быть использован в клинико-диагностических лабораториях медицинских учреждений.

1.7 Потенциальные пользователи: квалифицированный персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории: врач клинико-диагностической лаборатории, фельдшер-лаборант (медицинский лабораторный техник).

1.8 Применять набор реагентов строго по назначению согласно данной инструкции по применению.

2 ХАРАКТЕРИСТИКА НАБОРА РЕАГЕНТОВ

Набор реагентов для выявления ДНК Herpes Simplex Virus 1, Herpes Simplex Virus 2, Cytomegalovirus методом ПЦР в режиме реального времени (HSV1/HSV2/CMV ГерпесКомплекс) выпускается в стандартной фасовке (маркируется – фасовка S, стрипы; фасовка S, пробирки) и в универсальной фасовке для ручного и автоматизированного дозирования (маркируется – фасовка U).

2.1 Состав набора реагентов

REF R1-P211-S3/9, фасовка S, стрипы			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации, запечатанная парафином	Прозрачная бесцветная или розовая жидкость под воскообразным белым слоем	12 стрипов по 8 пробирок	по 20 мкл
Раствор Taq-полимеразы	Прозрачная бесцветная жидкость	2 пробирки	по 500 мкл
Минеральное масло	Прозрачная бесцветная вязкая маслянистая жидкость	2 пробирки	по 1,0 мл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл
Крышки для стрипов	12 шт		

REF R1-P211-23/9, фасовка S, пробирки			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации, запечатанная парафином	Прозрачная бесцветная или розовая жидкость под воскообразным белым слоем	96 пробирок	по 20 мкл
Раствор Taq-полимеразы	Прозрачная бесцветная жидкость	2 пробирки	по 500 мкл
Минеральное масло	Прозрачная бесцветная вязкая маслянистая жидкость	2 пробирки	по 1,0 мл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл

REF R1-P211-UA/9, фасовка U			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации	Прозрачная бесцветная или розовая жидкость	1 пробирка	600 мкл
Полимераза ТехноTaq MAX	Прозрачная бесцветная вязкая жидкость	1 пробирка	30 мкл
ПЦР-буфер	Прозрачная бесцветная жидкость	1 пробирка	600 мкл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл

[1] - на этикетке компонента для всех фасовок «Положительный контрольный образец» указывается как «К+»

Все компоненты набора реагентов готовы к применению и не требуют дополнительной подготовки к работе.

Комплектность:

- Набор реагентов в одном из вариантов исполнения – 1 шт.
- Инструкция по применению – 1 экз.
- Вкладыш – 1 экз.
- Паспорт – 1 экз.

2.2 Количество анализируемых образцов

Набор реагентов в фасовке S рассчитан на 96 определений (не более 24 постановок), включая анализ неизвестных образцов, отрицательных контрольных образцов и положительных контрольных образцов.

Набор реагентов в фасовке U рассчитан на проведение 96 определений при условии постановки не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

2.3 Принцип метода

Метод: Полимеразная цепная реакция (ПЦР) с детекцией результатов в режиме реального времени; мультиплексный качественный анализ.

Принцип метода основан на использовании процесса амплификации ДНК, заключающегося в повторяющихся циклах температурной денатурации ДНК, отжига праймеров с комплементарными последовательностями и последующей достройки полинуклеотидных цепей с этих праймеров Taq-полимеразой.

Для повышения чувствительности и специфичности реакции предусмотрено применение «горячего» старта. Для фасовки S «горячий» старт обеспечивается методикой приготовления реакционной смеси, состоящей из двух слоёв, разделённых прослойкой из парафина. Смешение слоёв и превращение их в амплификационную смесь происходит только после плавления парафина, что исключает неспецифическое связывание праймеров с ДНК-мишенью при начальном прогреве пробирки. «Горячий» старт для фасовки U обеспечивается использованием полимеразы, активность которой блокирована антителами, активация фермента происходит только после предварительного прогрева реакционной смеси при 94 °С. Это исключает неспецифическое связывание праймеров с ДНК-мишенью при начальном прогреве пробирки.

В смесь для амплификации введены ДНК-зонды, каждый из которых содержит флуоресцентную метку и гаситель флуоресценции. При образовании специфического продукта ДНК-зонд разрушается, действие гасителя на флуоресцентную метку прекращается, что ведёт к возрастанию уровня флуоресценции, который фиксируется детектирующим амплификатором. Количество разрушенных зондов (а, следовательно, и уровень флуоресценции) увеличивается пропорционально количеству образовавшихся специфических ампликонов. Уровень флуоресценции измеряется на каждом цикле амплификации в режиме реального времени.

В состав смеси для амплификации включен внутренний контроль (ВК), который предназначен для контроля прохождения полимеразной цепной реакции.

В состав ДНК-зонда, использующегося для детекции продукта амплификации ДНК Herpes simplex virus 2, включена флуоресцентная метка Fam. В состав ДНК-зонда, использующегося для детекции продукта амплификации ДНК Cytomegalovirus, включена флуоресцентная метка Rox. В состав ДНК-зонда, использующегося для детекции продукта амплификации ДНК Herpes simplex virus 1, включена флуоресцентная метка Cy5. В состав ДНК-зонда, использующегося для детекции продукта амплификации внутреннего контроля, входит флуоресцентный краситель Hex.

Использование нескольких флуоресцентных красителей позволяет сократить количество пробирок и биоматериала, необходимого для проведения исследования, поскольку появляется возможность одновременно регистрировать результаты разных реакций амплификации, проходящих в одной пробирке.

В таблице 1 приведены каналы детекции продуктов амплификации.

Таблица 1 – Каналы детекции продуктов амплификации

Fam/Green	Hex/Yellow/Vic	Rox/Orange	Cy5/Red	Cy5.5/Crimson
Herpes simplex virus 2 (HSV2)	ВК	Cytomegalovirus (CMV)	Herpes simplex virus 1 (HSV1)	-

Исследование состоит из следующих этапов: выделение ДНК (пробоподготовка), ПЦР-амплификация ДНК с одновременной детекцией результатов в режиме реального времени с использованием набора реагентов HSV1/HSV2/CMV ГерпесКомплекс.

3 АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1 Аналитическая специфичность

В образцах биологического материала, содержащих ДНК выявляемых микроорганизмов, при проведении амплификации программное обеспечение детектирующего амплификатора должно регистрировать положительные результаты амплификации специфических продуктов (фрагментов геномов Herpes simplex virus 1, Herpes simplex virus 2 или Cytomegalovirus) по заявленным каналам детекции.

В образцах биологического материала, не содержащих ДНК выявляемых микроорганизмов при проведении амплификации программное обеспечение детектирующего амплификатора должно регистрировать отрицательные результаты амплификации специфических продуктов (фрагментов геномов Herpes simplex virus 1, Herpes simplex virus 2 или Cytomegalovirus) по заявленным каналам детекции и положительный результат амплификации внутреннего контроля по каналу детекции Hex/Yellow/Vic.

Показано отсутствие неспецифических положительных результатов амплификации при наличии в образце ДНК Varicella-zoster virus, Epstein-Barr virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, *Staphylococcus* spp., *Streptococcus* spp., Parvovirus B19, *Chlamydia pneumonia, Mycoplasma hominis, Trichomonas vaginalis, Neisseria gonorrhoeae, Toxoplasma gondii, Mycoplasma genitalium, Chlamydia trachomatis, Gardnerella vaginalis,* а также ДНК человека в концентрации до $1{,}0\times10^8$ копий/мл образца.

3.2 Интерферирующие вещества

Наличие ингибиторов ПЦР в образце биологического материала может быть причиной сомнительных (неопределённых/недостоверных) результатов. Признаком ингибирования ПЦР является одновременное отсутствие амплификации внутреннего контроля и специфического продукта.

К ингибиторам ПЦР отнесены следующие вещества: гемоглобин и лекарственные препараты, присутствующие в образце ДНК в результате неполного удаления в процессе выделения ДНК из образца биоматериала, а также изопропиловый спирт и метилацетат, присутствующие в образце ДНК в результате неполного удаления промывочных растворов в ходе пробоподготовки.

Максимальные концентрации интерферирующих веществ, при которых не наблюдалось влияние на амплификацию лабораторных контрольных образцов и внутреннего контрольного образца составляют: гемоглобин – 0,35 мг/мл образца ДНК, изопропиловый спирт – 100 мкл/мл образца ДНК, метилацетат – 100 мкл/мл образца ДНК.

Для оценки возможной интерференции лекарственных препаратов были выбраны те, которые потенциально могут присутствовать в остаточных количествах в биологических образцах человека, взятых из соответствующих исследуемых биотопов (Мирамистин®, хлоргексидин биглюконат).

Для всех исследуемых лекарственных препаратов было показано отсутствие их влияния в концентрации до 10% в образце биоматериала.

3.3 Предел обнаружения

Предел обнаружения составляет по 5 копий ДНК каждого микроорганизма на амплификационную пробирку.

Предел обнаружения установлен путём анализа серийных разведений двух серий лабораторных контрольных образцов (ЛКО).

Предел обнаружения соответствует следующим значениям концентрации ДНК при использовании указанных наборов/комплектов реагентов для выделения ДНК и конечного объёма элюции (разведения) выделенной ДНК:

Биоматериал	Наименование набора/комплекта реагентов для выделения ДНК	Объём полученного препарата, мкл	Предел обнаружения, копий/образец
Соскобы эпителиальных клеток в 500 мкл транспортной среды[1], моча (1,0 мл)	ПРОБА-НК	50	50
	ПРОБА-НК-ПЛЮС	300	300
	ПРОБА-ГС	100	100
	ПРОБА-ГС-ПЛЮС	300	300
	ПРОБА-РАПИД	500	500
	ПРОБА-МЧ-РАПИД[2]	100	100
	ПРОБА-МЧ-РАПИД II	100	100
	ПРОБА-ОПТИМА	400	400

3.4 Диагностические характеристики

Вид биоматериала	Аналит	Диагностическая чувствительность	Диагностическая специфичность
Соскобы эпителиальных клеток из урогенитального тракта	HSV1	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)
	HSV2	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)
	CMV	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)
Моча	HSV1	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)
	HSV2	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)
	CMV	100% (95% ДИ: 86,28% – 100%)	100% (95% ДИ: 86,28% – 100%)

3.5 Воспроизводимость и повторяемость

Воспроизводимость составляет 100%.

Повторяемость составляет 100%.

[1] - в качестве транспортной среды использовалась Транспортная среда для биопроб СТОР-Ф, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2020/9640

[2] - только для соскобов эпителиальных клеток

4 МЕРЫ ПРЕДОСТОРОЖНОСТИ

Организация работы ПЦР-лаборатории, оборудование и материалы должны соответствовать требованиям ГОСТ Р ИСО 15190-2023, методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот, при работе с материалом, содержащим микроорганизмы I-IV групп патогенности», с соблюдением санитарных правил и норм СанПиН 3.3686-21 «Санитарно-эпидемиологические требования по профилактике инфекционных болезней».

Неизвестные образцы рассматриваются как потенциально-опасные. При работе с набором реагентов следует надевать одноразовые перчатки без талька.

При работе с микроорганизмами I-IV групп патогенности выбор типа защитного костюма (рабочей одежды и средств индивидуальной защиты) проводится в строгом соответствии с санитарными правилами и нормами СанПиН 3.3686-21 и определяется видом возбудителя, рабочей зоной, оснащением ее боксами биологической безопасности.

Следует использовать только одноразовые наконечники и пробирки.

Не допускается использование одних и тех же наконечников при обработке различных образцов биологического материала.

К работе с набором реагентов допускается персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории.

Выделение ДНК следует проводить в боксах биологической безопасности II класса. Подготовку к ПЦР с использованием набора реагентов возможно проводить в ПЦР-боксах.

Запрещается перемещение лабораторного оборудования, в том числе дозаторов, штативов, лабораторной посуды, халатов, головных уборов и пр., а также растворов реагентов из одного помещения в другое.

Дозаторы должны быть соответствующим образом поверены (в аккредитованных лабораториях) и промаркированы.

Использованные одноразовые принадлежности (пробирки, наконечники и др.) должны сбрасываться в контейнер для медицинских отходов, содержащий дезинфицирующий раствор (при необходимости).

Поверхности рабочих столов, а также помещения, в которых проводится выделение НК и постановка ПЦР, следует обязательно, до и после проведения работ, облучать с помощью бактерицидных установок в течение 30 минут.

Все поверхности в лаборатории (рабочие столы, штативы, оборудование и др.) ежедневно подвергают влажной уборке с применением дезинфицирующих/моющих средств, регламентированных санитарными правилами и нормами СанПиН 3.3686-21.

ВНИМАНИЕ! Утилизировать отходы с продуктами ПЦР необходимо только в закрытом виде. Не допускается открывать пробирки после амплификации, так как это может привести к контаминации продуктами ПЦР (МУ 1.3.2569-09).

При использовании набора реагентов в клинико-диагностической лаборатории образуются отходы класса В, которые утилизируются в соответствии с требованиями санитарных правил и норм СанПиН 2.1.3684-21 «Санитарно-эпидемиологические требования к содержанию территорий городских и сельских поселений, к водным объектам, питьевой воде и питьевому водоснабжению, атмосферному воздуху, почвам, жилым помещениям, эксплуатации производственных, общественных помещений, организации и проведению санитарно-противоэпидемических (профилактических) мероприятий».

Опасные компоненты в наборе реагентов

Компонент набора реагентов	**Наличие/отсутствие опасного компонента**		**Указание на риски**
	Фасовка S	Фасовка U	
Смесь для амплификации, запечатанная парафином	Нет опасных веществ	-	-
Раствор Taq-полимеразы	Нет опасных веществ	-	-
Минеральное масло	Нет опасных веществ	-	-
Смесь для амплификации	-	Нет опасных веществ	-
Полимераза ТехноТаq MAX	-	Нет опасных веществ	-
ПЦР-буфер	-	Нет опасных веществ	-
Положительный контрольный образец	**Азид натрия менее 0,1%**	**Азид натрия менее 0,1%**	Не классифицируется как опасный для здоровья человека и окружающей среды

При работе с набором реагентов следует использовать средства индивидуальной защиты для предотвращения контакта с организмом человека. После окончания работы тщательно вымыть руки. Избегать контакта с кожей, глазами и слизистыми оболочками

При использовании по назначению и соблюдении мер предосторожности, контакт с организмом человека исключен.

Не использовать набор реагентов:

- при нарушении условий транспортирования и хранения;
- при несоответствии внешнего вида реагентов, указанного в паспорте к набору реагентов;
- при нарушении внутренней упаковки компонентов набора реагентов;
- по истечению срока годности набора реагентов.

Примечание – Набор реагентов **не содержит** материалов биологического происхождения, веществ в концентрациях, обладающих канцерогенным, мутагенным действием, а также влияющих на репродуктивную функцию человека. При использовании по назначению и соблюдении мер предосторожности является безопасным.

5 ОБОРУДОВАНИЕ И МАТЕРИАЛЫ

При работе с набором реагентов требуются следующие оборудование, реагенты и расходные материалы:

Оборудование, реагенты и расходные материалы	Фасовка S		Фасовка U, дозирование	
	стрипы[1]	пробирки	ручное	автомати-зированное
ПЦР-бокс	да	да	да	да
амплификатор с детекцией в режиме реального времени[2]	да	да	да	да[3]
микроцентрифуга-вортекс	да	да	да	да
ротор для микроцентрифуги-вортекса для стрипованных пробирок объёмом 0,2 мл	да	нет	нет	нет
холодильник с морозильной камерой	да	да	да	да
штатив «рабочее место» для пробирок объёмом 1,5 мл	да	да	да	да
штатив «рабочее место» для пробирок объёмом 0,2 мл	нет	да	да[4]	нет
штатив «рабочее место» для стрипованных пробирок объёмом 0,2 мл	да	нет	нет	нет
дозаторы механические или электронные одноканальные с переменным объёмом, позволяющие отбирать объём жидкости от 0,5 до 10 мкл, от 2,0 до 20 мкл, от 20 до 200 мкл, от 200 до 1000 мкл	да	да	да	да
одноразовые наконечники с фильтром для полуавтоматических дозаторов, свободные от РНКаз и ДНКаз, объёмом 10 мкл, 20 мкл, 200 мкл, 1000 мкл	да	да	да	да
штатив для дозаторов	да	да	да	да
пробирки микроцентрифужные объёмом 1,5 мл с крышками, свободные от РНКаз и ДНКаз	нет	нет	да	да
пробирки амплификационные объёмом 0,2 мл с крышками, свободные от РНКаз и ДНКаз или микропланшет ПЦР 96 лунок[5]	нет	нет	да	нет
одноразовые перчатки медицинские, без талька, текстурированные	да	да	да	да
ёмкость для сброса использованных наконечников, пробирок и других расходных материалов	да	да	да	да
Устройство дозирующее ДТстрим по ТУ 9443-005-96301278-2012 в варианте исполнения ДТстрим 12М1 или ДТстрим 15М1, ООО «НПО ДНК-Технология», Россия, РУ № РЗН 2015/2982, далее по тексту – ДТстрим	нет	нет	нет	да
одноразовые наконечники с фильтром для дозирующего устройства ДТстрим в комплектации *М1, свободные от РНКаз и ДНКаз, объёмом 200 мкл или рекомендованные для аналогичного используемого дозирующего устройства	нет	нет	нет	да
Устройство для запечатывания планшетов ДТпак, ООО «НПО ДНК-Технология», Россия	нет	нет	да[6]	да
центрифуга с RCF(g) не ниже 100, с адаптером для микропланшетов	нет	нет	да[6]	да
полимерная термоплёнка для запечатывания микропланшетов ПЦР	нет	нет	да[6]	да
микропланшет 384 лунки	нет	нет	нет	да
транспортная среда (при необходимости), рекомендуется использовать Транспортную среду для биопроб СТОР-Ф по ТУ 21.20.23-101-46482062-2019, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2020/9640 или Транспортную среду для биопроб с муколитиком (СТОР-М) по ТУ 21.20.23-102-46482062-2019, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2019/9453 (для соскобов эпителиальных клеток из урогенитального тракта)				
физиологический раствор (0,9% NaCl) стерильный (при необходимости)				

Оборудование, реагенты и расходные материалы	Фасовка S		Фасовка U, дозирование	
	стрипы[1]	пробирки	ручное	автомати-зированное
набор/комплект реагентов для выделения НК из биологического материала[7], рекомендуются: - Комплект реагентов для выделения нуклеиновых кислот (ПРОБА-НК/ПРОБА-НК-ПЛЮС) по ТУ 9398-035-46482062-2009 в формах комплектации: комплект ПРОБА-НК, комплект ПРОБА-НК-ПЛЮС, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2010/08867; - Комплект реагентов для выделения ДНК по ТУ 9398-037-46482062-2009 в формах комплектации ПРОБА-ГС и ПРОБА-ГС-ПЛЮС, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2010/08696; - Комплект реагентов для выделения ДНК (ПРОБА-РАПИД) по ТУ 9398-015-46482062-2008, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2008/02939; - Набор реагентов для выделения нуклеиновых кислот (ПРОБА-МЧ) по ТУ 9398-088-46482062-2016 в комплектации ПРОБА-МЧ-РАПИД, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2017/5753; - Набор реагентов для выделения ДНК человека, бактерий, вирусов и грибов из биологического материала человека и культур микроорганизмов (ПРОБА ОПТИМА) по ТУ 21.20.23-124-46482062-2021, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2022/17496; - Набор реагентов для выделения ДНК/РНК человека, бактерий, вирусов и грибов из биологического материала человека (ПРОБА-МЧ-РАПИД II) по ТУ 21.20.23-136-46482062-2023, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2024/23205.				
Примечания к таблице: [1] - не используется для детектирующего амплификатора Rotor-Gene Q [2] - далее по тексту – детектирующий амплификатор; требуемые параметры детектирующих амплификаторов указаны ниже [3] - валидирован детектирующий амплификатор «ДТпрайм» (модификация ДТпрайм» *X*), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229 [4] - только при использовании пробирок [5] - не используется для детектирующих амплификаторов «ДТлайт» и Rotor-Gene Q [6] - только при использовании микропланшетов [7] - возможность использования набора/комплекта реагентов для выделения ДНК определяется видом биологического материала (7.1)				

Набор реагентов применяется с детектирующими амплификаторами планшетного и роторного типа с системой детекции флуоресцентного сигнала в режиме реального времени, зарегистрированными в установленном порядке в РФ и соответствующими следующим требованиям:

- обеспечивается работа с объемом реакционной смеси 35 мкл (фасовка S) или 18 мкл (фасовка U);
- обеспечивается работа с флуорофорами: Fam, Hex (Vic), Rox, Cy5;
- подогреваемая крышка с температурой более 100 °С;
- скорость нагрева не менее 2 °С/с;
- скорость охлаждения не менее 1 °С/с;
- точность поддержания и однородность температуры не более ± 0,4 °С.

Для работы с набором реагентов валидированы следующие детектирующие амплификаторы:

- Амплификатор детектирующий «ДТпрайм» по ТУ 9443-004-96301278-2010 (модификация «ДТпрайм *M*»), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229, далее по тексту – «ДТпрайм»;
- Амплификатор детектирующий «ДТпрайм» по ТУ 9443-004-96301278-2010 (модификация «ДТпрайм *X*») ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229 (только для набора реагентов в фасовке U для автоматизированного дозирования), далее по тексту – «ДТпрайм» в модификации «ДТпрайм *X*»;

- Амплификатор детектирующий «ДТлайт» по ТУ 9443-003-96301278-2010 (модификация «ДТлайт *S*»), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10228 (только для набора реагентов в фасовке S; в фасовке U для ручного дозирования при использовании пробирок), далее по тексту – «ДТлайт»;
- Прибор для проведения полимеразной цепной реакции в режиме реального времени Rotor-Gene Q, QIAGEN GmbH, Германия, РУ № ФСЗ 2010/07595 (только для набора реагентов в фасовке S, пробирки), далее по тексту – Rotor-Gene Q;
- Термоциклер для амплификации нуклеиновых кислот 1000 с модулем реакционным оптическим CFX96 (Optical Reaction Module CFX96), Био-Рад Лабораториез, Инк; США, РУ № ФСЗ 2008/03399, далее по тексту – CFX96;
- Амплификатор нуклеиновых кислот Applied Biosystems QuantStudio 5 с гибридизационно-флуоресцентной детекцией продуктов ПЦР в режиме реального времени, «Лайф Текнолоджис Холдингс Пте. Лтд.», Сингапур, РУ № РЗН 2019/8446, далее по тексту – Applied Biosystems QuantStudio 5.

По вопросам применения детектирующих амплификаторов, не указанных выше, требуется согласование с производителем набора реагентов.

6 АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1 Материал для исследования

Для исследования используют мочу, соскобы эпителиальных клеток из урогенитального тракта.

6.2 Общие требования

6.2.1 Исследование методом ПЦР относится к прямым методам лабораторного исследования, поэтому взятие биологического материала человека необходимо проводить из места локализации инфекционного процесса. Решение о необходимости исследовать ту или иную локализацию принимает лечащий врач на основании собранного анамнеза и клинической картины заболевания.

6.2.2 Для получения корректных результатов большое значение имеет качество взятия образца биоматериала для исследования, его хранение, транспортирование и предварительная обработка.

Неправильное взятие биоматериала может привести к получению недостоверных результатов и, вследствие этого, необходимости его повторного взятия.

6.2.3 При необходимости взятия соскобов из нескольких биотопов повторите процедуру, каждый раз забирая материал в новую пробирку.

6.2.4 На этапе подготовки биоматериала используйте одноразовые наконечники с фильтром, свободные от РНКаз и ДНКаз.

6.2.5 Для предотвращения контаминации открывайте крышку только той пробирки, в которую будете вносить биологический материал, и закрывайте ее перед работой со следующей пробиркой.

Примечание – Взятие, предварительную обработку, хранение и перевозку, передачу исследуемого материала в другие организации осуществляют согласно инструктивно-методическим документам, регламентирующим выполнение исследований в соответствии с требованиями МУ 1.3.2569-09 и СанПиН 3.3686-21.

6.3 Взятие материала на исследование

ВНИМАНИЕ! Перед выделением ДНК может потребоваться подготовка образцов биологического материала (6.5).

6.3.1 Моча

Взятие материала проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

6.3.2 Соскобы эпителиальных клеток из урогенитального тракта

Взятие материала осуществляют с помощью специальных медицинских изделий, имеющих регистрационные удостоверения, согласно установленной в зависимости от источника биологического материала процедуре (например, Зонды медицинские по ТУ 9436-002-98349125-2016 в вариантах исполнения: 1. Зонд тип А универсальный: – тип А1, производство ООО «Медицинские изделия», Россия, РУ № РЗН 2018/7058).

ВНИМАНИЕ! Взятие материала в пробирки с реактивом «ПРОБА-РАПИД» осуществляется сухим зондом! Необходимо исключить контакт раствора с кожей, глазами и слизистыми оболочками.

Ограничение метода[1]: местное применение лекарственных препаратов, УЗИ вагинальным датчиком, кольпоскопия – менее чем за 24 часа до исследования.

Взятие материала проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

6.3.2.1 Особенности взятия урогенитальных соскобов

Женщины накануне обследования не должны проводить туалет половых органов и спринцевание. Для получения объективного результата необходимо, чтобы исследуемый материал содержал возможно большее количество эпителиальных клеток и минимальное количество слизи и примеси крови.

ВНИМАНИЕ! Перед получением соскоба эпителиальных клеток из уретры, с заднего свода влагалища и цервикального канала свободно стекающее отделяемое необходимо удалить стерильным ватным тампоном.

При необходимости взятия биоматериала из нескольких биотопов повторите процедуру, каждый раз забирая материал новым зондом в новую пробирку.

6.3.2.2 Особенности взятия материала из влагалища

Материал должен быть взят до проведения мануального исследования. Зеркало перед манипуляцией можно смочить горячей водой, применение антисептиков для обработки зеркала противопоказано. Соскоб берут с заднебокового свода влагалища. У девочек взятие материала производят со слизистой оболочки преддверия влагалища, а в отдельных случаях – из заднего свода влагалища через гименальные кольца.

6.3.2.3 Особенности взятия материала из уретры

Перед взятием биоматериала пациенту рекомендуется воздержаться от мочеиспускания в течение 1,5–2 часов.

Непосредственно перед взятием биоматериала необходимо обработать наружное отверстие уретры тампоном, который можно смочить стерильным физиологическим раствором.

При наличии гнойных выделений соскоб рекомендуется брать через 15-20 минут после мочеиспускания, при отсутствии выделений необходимо провести массаж уретры с помощью зонда для взятия биоматериала. В уретру у женщин зонд вводится на глубину 1,0-1,5 см, у детей материал для исследования берут только с наружного отверстия уретры.

[1] - если это не противоречит требованиям к используемым наборам/комплектам реагентов для выделения НК

6.3.2.4 Особенности взятия материала из цервикального канала

Перед взятием материала необходимо удалить ватным тампоном слизь и затем обработать шейку матки стерильным физиологическим раствором. Зонд вводят в цервикальный канал на глубину 0,5-1,5 см. При извлечении зонда необходимо полностью исключить его касание стенок влагалища.

6.4 Транспортирование и хранение образцов биологического материала

6.4.1 Моча

Условия транспортирования и хранения образцов мочи определяются инструкциями по применению рекомендуемых наборов/комплектов реагентов для выделения ДНК (7.1) или используемых для транспортирования и хранения образцов транспортных сред.

Образцы мочи допускается транспортировать и хранить (если это не противоречит требованиям к используемым наборам/комплектам реагентов для выделения НК):

- при температуре от 2 °С до 8 °С – не более одних суток;
- при температуре от минус 22 °С до минус 18 °С – не более одной недели.

ВНИМАНИЕ! Допускается лишь однократное замораживание-оттаивание материала.

6.4.2 Соскобы эпителиальных клеток из урогенитального тракта

Условия транспортирования и хранения соскобов из урогенитального тракта определяются инструкциями по применению рекомендуемых наборов/комплектов реагентов для выделения ДНК (7.1) или используемых для транспортирования и хранения образцов транспортных сред.

Допускается хранение образцов при температуре от 2 °С до 8 °С не более 24 ч. В случае невозможности доставки материала в лабораторию в течение суток допускается однократное замораживание материала. Допускается хранение замороженного материала при температуре от минус 22 °С до минус 18 °С в течение одного месяца (если это не противоречит требованиям к используемым наборам/комплектам реагентов для выделения НК).

ВНИМАНИЕ! Допускается лишь однократное замораживание-оттаивание материала.

6.5 Подготовка биологического материала человека для выделения ДНК

Подготовка биологического материала (при необходимости) проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

7 ПРОВЕДЕНИЕ АНАЛИЗА

7.1 Выделение ДНК из биологического материала

Для выделения ДНК рекомендуется использовать наборы/комплекты реагентов, имеющие регистрационные удостоверения на медицинское изделие и предназначенные для соответствующих видов биоматериала с целью последующего исследования ДНК методом ПЦР, например, ПРОБА-РАПИД (не рекомендуется для соскобов из урогенитального тракта у мужчин), ПРОБА-НК, ПРОБА-НК-ПЛЮС, ПРОБА-ГС, ПРОБА-ГС-ПЛЮС, ПРОБА-МЧ-РАПИД, ПРОБА-ОПТИМА, ПРОБА-МЧ-РАПИД II (таблица 2).

Таблица 2 – Наборы/комплекты реагентов, рекомендованные для выделения ДНК для дальнейшего исследования с использованием набора реагентов HSV1/HSV2/CMV ГерпесКомплекс

Набор/комплект реагентов, РУ	Биоматериал	Минимальное количество элюата, мкл
Комплект реагентов ПРОБА-НК, РУ № ФСР 2010/08867	Моча, соскобы эпителиальных клеток из урогенитального тракта	50
Комплект реагентов ПРОБА-НК-ПЛЮС, РУ № ФСР 2010/08867	Моча, соскобы эпителиальных клеток из урогенитального тракта	300
Комплект реагентов ПРОБА-ГС, РУ № ФСР 2010/08696	Моча, соскобы эпителиальных клеток из урогенитального тракта	100
Комплект реагентов ПРОБА-ГС-ПЛЮС, РУ № ФСР 2010/08696	Моча, соскобы эпителиальных клеток из урогенитального тракта	300
Набор реагентов ПРОБА-МЧ-РАПИД, РУ № РЗН 2017/5753	Соскобы эпителиальных клеток из урогенитального тракта	100
Комплект реагентов ПРОБА-РАПИД, РУ № ФСР 2008/02939	Моча, соскобы эпителиальных клеток из урогенитального тракта	500
Набор реагентов ПРОБА-ОПТИМА, РУ № РЗН 2022/17496	Моча, соскобы эпителиальных клеток из урогенитального тракта	400
Набор реагентов ПРОБА-МЧ-РАПИД II, РУ № РЗН 2024/23205	Моча, соскобы эпителиальных клеток из урогенитального тракта	100

Выделение ДНК из исследуемого материала проводят в соответствии с инструкцией по применению используемого набора/комплекта реагентов.

ВНИМАНИЕ! Одновременно с выделением ДНК из биологического материала необходимо подготовить отрицательный контрольный образец и провести его через все этапы пробоподготовки. Для этого рекомендуется использовать физиологический раствор или отрицательный контрольный образец, входящий в состав набора/комплекта реагентов для выделения нуклеиновых кислот, в объёме, указанном в инструкции по применению соответствующего набора/комплекта реагентов.

7.2 Подготовка и проведение ПЦР. Фасовка S

ВНИМАНИЕ!

1. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!
2. При использовании набора реагентов в варианте исполнения «Фасовка S, стрипы» следует строго соблюдать комплектность стрипов и крышек к ним. Не использовать крышки к стрипам из других наборов реагентов!

7.2.1 Промаркируйте по одной пробирке/стрипованной пробирке со смесью для амплификации, запечатанной парафином, для каждого неизвестного образца, для отрицательного контрольного образца (К-) и для положительного контрольного образца (К+).

ВНИМАНИЕ! Количество реагентов рассчитано не более чем на 24 постановки при условии вариабельного количества неизвестных образцов, 1 отрицательного контрольного образца и 1 положительного контрольного образца в каждой постановке.

Пример:

Необходимо проанализировать 4 неизвестных образца. Для этого нужно промаркировать 4 пробирки для неизвестных образцов, одну пробирку для «К-» и одну пробирку для «К+». Общее количество пробирок – 6.

7.2.2 Встряхните пробирку с раствором Taq-полимеразы на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.2.3 Добавьте во все промаркированные пробирки, не повреждая слой парафина, по 10 мкл раствора Taq-полимеразы.

ВНИМАНИЕ! При использовании для проведения ПЦР детектирующего амплификатора Rotor-Gene Q минеральное масло в пробирки не вносится!

7.2.4 Добавьте в каждую пробирку (при необходимости) по одной капле (около 20 мкл) минерального масла. Неплотно прикройте пробирки/стрипы крышками.

7.2.1. Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Для препарата ДНК и отрицательного контрольного образца перед внесением в пробирки с реакционной смесью необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-РАПИД, ПРОБА-НК, ПРОБА-НК-ПЛЮС, ПРОБА-ГС и ПРОБА-ГС-ПЛЮС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) необходимо встряхнуть пробирки с препаратом ДНК и отрицательным

контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, необходимо, не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

4. Для предотвращения контаминации следует перед внесением ДНК открывать крышки только тех пробирок, в которые будет вноситься данный образец, и закрывать их, перед внесением следующего. В случае использования стрипов следует закрывать крышку стрипа после внесения в него образцов перед началом работы со следующим. Необходимо закрывать пробирки/стрипы плотно. Препараты ДНК и контрольные образцы следует вносить наконечниками с фильтром.

7.2.5 Внесите в соответствующие промаркированные пробирки, не повреждая слой парафина, по 5,0 мкл выделенного из образцов препарата ДНК. В пробирки, промаркированные «К-» и «К+», ДНК не вносится.

7.2.6 Внесите в пробирку, промаркированную «К-», не повреждая слой парафина, 5,0 мкл отрицательного контрольного образца, прошедшего этап выделения ДНК (см. 7.1).

7.2.7 Внесите в пробирку, промаркированную «К+», не повреждая слой парафина, 5,0 мкл положительного контрольного образца.

7.2.8 Центрифугируйте все пробирки/стрипы на микроцентрифуге-вортексе в течение 3-5 с (при использовании для проведения ПЦР детектирующего амплификатора Rotor-Gene Q центрифугирование не обязательно).

7.2.9 Установите все пробирки/стрипы в детектирующий амплификатор.

7.2.10 Для детектирующих амплификаторов серии ДТ:

Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительного контрольных образцов, отметьте расположение пробирок/стрипов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 3.

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении А) или предоставляется производителем набора реагентов

7.2.11 Для детектирующих амплификаторов CFX96, Applied Biosystems QuantStudio 5 и Rotor-Gene Q:

Проведите ПЦР с учетом объёма реакционной смеси, равного 35 мкл, по программам амплификации, приведённым в таблицах 4, 5, 6 соответственно.

Таблица 3 – Программа амплификации для детектирующих амплификаторов «ДТпрайм», «ДТлайт» (фасовка S)

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	30	1		Цикл
	94	1	30			
2	94	0	30	5		Цикл
	64	0	15		√	
3	94	0	10	45		Цикл
	64	0	15		√	
4	94	0	5	1		Цикл
5	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

Таблица 4 – Программа амплификации для детектирующих амплификаторов CFX96 (фасовки S, U)

№ блока (Step)	Температура, °С	Время, мин: сек	Количество циклов (повторов)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ - режим оптических измерений (Plate Read), установить измерение флуоресценции по необходимым каналам детекции (Fam, Hex, Rox, Cy5) при 64 °С			

Таблица 5 – Программа амплификации для детектирующих амплификаторов Applied Biosystems QuantStudio 5 (фасовки S, U)

Стадия	№ шага	Температура, °С	Время, мин: сек	Количество циклов (повторов)
Стадия удержания	1	80	01:00	1
	2	94	01:30	1
Стадия ПЦР	1	94	0:20	50
	2	62 √	0:20	
√ - сбор данных для необходимых флуорофоров (Fam, Vic (Hex), Rox, Cy5) включен				

[1] - допускается хранение при температуре 10 °С

Таблица 6 – Программа амплификации для детектирующего амплификатора Rotor-Gene Q (фасовка S, пробирки)

№/ Cycling	Температура/ Temperature	Время/ Hold Time	Количество циклов/ Cycle Repeats
Cycling 1	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	62 deg √	15 sec	
Cycling 3	94 deg	10 sec	45 times
	62 deg √	15 sec	
√ - режим оптических измерений установить измерение флуоресценции (Acquiring) по каналам детекции Green, Yellow, Orange и Red при 62 °C			

7.3 Подготовка и проведение ПЦР. Фасовка U, ручное дозирование

ВНИМАНИЕ!

1. Для амплификации следует использовать одноразовые амплификационные пробирки объёмом 0,2 мл или микропланшеты ПЦР 96 лунок[1], герметизируемые термоплёнкой. Не рекомендуется использовать стрипованные пробирки в связи с опасностью постамплификационной контаминации.
2. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!

7.3.1 Промаркируйте необходимое количество одноразовых амплификационных пробирок объёмом 0,2 мл или микропланшет ПЦР 96 лунок для неизвестных образцов, для отрицательного контрольного образца (К-) и для положительного контрольного образца (К+).

Примечание – Рекомендуется постановка не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

Пример:

Необходимо проанализировать 4 неизвестных образца. Для этого нужно промаркировать 4 пробирки/зарезервировать 4 лунки микропланшета для неизвестных образцов, одну пробирку/лунку для «К-» и одну пробирку/лунку для «К+». Общее количество пробирок/лунок – 6.

7.3.2 Встряхните пробирку со смесью для амплификации на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.3.3 Внесите во все промаркированные пробирки/необходимые лунки микропланшета (включая «К–» и «К+») по 6,0 мкл смеси для амплификации.

7.3.4 Встряхните пробирки с ПЦР-буфером и полимеразой ТехноТаq MAX на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

[1] - для детектирующих амплификаторов «ДТлайт» микропланшеты 96 лунок не используются

ВНИМАНИЕ! Полимеразу ТехноТаq МАХ необходимо доставать из морозильной камеры непосредственно перед использованием.

7.3.5 Приготовьте смесь ПЦР-буфера с полимеразой ТехноТаq МАХ. Для этого смешайте в отдельной одноразовой пробирке:

- 6,0 х (N+1) мкл ПЦР-буфера,
- 0,3 х (N+1) мкл полимеразы ТехноТаq МАХ,

где N – количество промаркированных пробирок/количество необходимых лунок микропланшета с учётом «К-», «К+».

Пример:

Необходимо проанализировать 4 неизвестных образца, «К-», «К+». Промаркированных пробирок/необходимых лунок микропланшета – 6. Нужно приготовить смесь ПЦР-буфера и полимеразы ТехноТаq МАХ для 7 (6+1) пробирок/лунок, т.е. 42 мкл ПЦР-буфера + 2,1 мкл полимеразы ТехноТаq МАХ.

7.3.6 Встряхните пробирку с приготовленной смесью ПЦР-буфера и полимеразы ТехноТаq МАХ на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Смесь ПЦР-буфера и полимеразы ТехноТаq МАХ необходимо готовить непосредственно перед использованием.

7.3.7 Добавьте во все промаркированные пробирки/необходимые лунки микропланшета со смесью для амплификации по 6,0 мкл смеси ПЦР-буфера и полимеразы ТехноТаq МАХ. Неплотно закройте пробирки.

ВНИМАНИЕ! После добавления смеси ПЦР-буфера и полимеразы ТехноТаq МАХ в пробирки/лунки со смесью для амплификации необходимо в течение двух часов выполнить 7.3.8 – 7.3.14.

7.3.8 Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Для препарата ДНК и отрицательного контрольного образца перед внесением в пробирки/лунки с реакционной смесью необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-РАПИД, ПРОБА-НК, ПРОБА-НК-ПЛЮС, ПРОБА-ГС и ПРОБА-ГС-ПЛЮС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) необходимо встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, необходимо, не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

4. Для предотвращения контаминации следует перед внесением ДНК открывать крышки только тех пробирок, в которые будет вноситься данный образец, и закрывать их перед внесением следующего. Необходимо закрывать пробирки плотно. Препараты ДНК и контрольные образцы следует вносить наконечниками с фильтром.

7.3.9 Внесите в соответствующие промаркированные пробирки/необходимые лунки микропланшета по 6,0 мкл выделенного из образцов препарата ДНК. В пробирки/лунки, промаркированные «К-» и «К+», ДНК не вносится.

7.3.10 Внесите в пробирку/лунку, промаркированную «К-», 6,0 мкл отрицательного контрольного образца, прошедшего этап выделения ДНК (см.7.1).

7.3.11 Внесите в пробирку/лунку, промаркированную «К+», 6,0 мкл положительного контрольного образца.

7.3.12 В случае использования микропланшетов ПЦР 96 лунок:

7.3.12.1 Поместите аккуратно, не встряхивая, микропланшет ПЦР в подложку устройства для запечатывания планшетов ДТпак.

7.3.12.2 Проведите запечатывание микропланшета ПЦР полимерной термоплёнкой согласно руководству по эксплуатации прибора ДТпак.

7.3.12.3 Центрифугируйте микропланшет ПЦР при RCF(g) 100 в течение 30 с.

7.3.13 В случае использования пробирок:

Центрифугируйте все пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.3.14 Установите все пробирки/микропланшет ПЦР в блок детектирующего амплификатора и проведите ПЦР (7.3.15, 7.3.16).

7.3.15 Для детектирующих амплификаторов серии ДТ:

Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительного контрольных образцов, отметьте расположение образцов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 7.

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении Б) или предоставляется производителем набора реагентов

7.3.16 Для детектирующих амплификаторов CFX96 и Applied Biosystems QuantStudio 5: Проведите ПЦР с учетом объёма реакционной смеси, равного 18 мкл, по программам амплификации, приведённым в таблицах 4, 5 соответственно.

Таблица 7 – Программа амплификации для детектирующих амплификаторов «ДТпрайм», «ДТлайт» (фасовка U)

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	5	15		Цикл
	94	0	5			
2	94	5	00	1		Цикл
3	94	0	30	5		Цикл
	64	0	15		√	
4	94	0	10	45		Цикл
	64	0	15		√	
5	94	0	5	1		Цикл
6	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

7.4 Подготовка и проведение ПЦР. Фасовка U, с использованием дозирующего устройства ДТстрим (только для детектирующего амплификатора «ДТпрайм» в модификации «ДТпрайм *X*»)

ВНИМАНИЕ!

1. Для амплификации следует использовать микропланшеты ПЦР 384 лунки, герметизируемые термоплёнкой.
2. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!

Примечание – Рекомендуется постановка не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

7.4.1 Встряхните пробирку со смесью для амплификации на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.4.2 Встряхните пробирки с ПЦР-буфером и полимеразой ТехноТаq MAX на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

[1] - допускается хранение при температуре 10 °С

ВНИМАНИЕ! Полимеразу ТехноТаq МАХ необходимо доставать из морозильной камеры непосредственно перед использованием.

7.4.3 Следуя указаниям ПО дозирующего устройства ДТстрим, приготовьте в отдельной одноразовой пробирке смесь ПЦР-буфера и полимеразы ТехноТаq МАХ.

7.4.4 Встряхните пробирку с приготовленной смесью ПЦР-буфера и полимеразы ТехноТаq МАХ на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.4.5 Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Перед проведением дозирования для препарата ДНК и отрицательного контрольного образца необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-РАПИД, ПРОБА-НК, ПРОБА-НК-ПЛЮС, ПРОБА-ГС и ПРОБА-ГС-ПЛЮС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) необходимо встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.
3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, необходимо, не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

7.4.6 Установите пробирки со смесью для амплификации, со смесью ПЦР-буфера и полимеразы ТехноТаq МАХ, пробирки или глубоколуночные планшеты с препаратами ДНК, отрицательным контрольным образцом и положительным контрольным образцом, а также микропланшет ПЦР на рабочий стол ДТстрим и проведите дозирование компонентов согласно руководству по эксплуатации.

7.4.7 Поместите аккуратно, не встряхивая, микропланшет ПЦР в подложку устройства для запечатывания планшетов ДТпак после завершения программы на дозирующем устройстве ДТстрим.

7.4.8 Проведите запечатывание микропланшета ПЦР полимерной термоплёнкой согласно руководству по эксплуатации прибора ДТпак.

7.4.9 Центрифугируйте микропланшет ПЦР при RCF(g) 100 в течение 30 с.

7.4.10 Установите микропланшет ПЦР в блок детектирующего амплификатора.

7.4.11 Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительного контрольных образцов, отметьте расположение образцов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 6.

8 РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ АМПЛИФИКАЦИИ

Регистрация сигнала флуоресценции проводится детектирующим амплификатором автоматически во время амплификации.

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении Б) или предоставляется производителем набора реагентов

9 УЧЁТ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

9.1 Учет результатов амплификации осуществляется автоматически с помощью программного обеспечения, поставляемого с детектирующим амплификатором.

9.2 При использовании детектирующих амплификаторов CFX96 следует использовать регрессионный тип анализа (Cq Determination Mode: Regression), во вкладке «Baseline Subtraction» необходимо выбрать «Baseline Subtraction Curve Fit».

9.3 Интерпретация результатов проводится в соответствии с таблицей 8. Результаты постановки валидны, если выполняются условия интерпретации результатов, полученных для контрольных образцов.

Таблица 8 – Интерпретация результатов ПЦР

Канал детекции				**Интерпретация результата**
Fam/Green, Cp/Cq/Ct	Hex/Yellow/Vic, Cp/Cq/Ct	Rox/Orange, Cp/Cq/Ct	Cy5/Red, Cp/Cq/Ct	
Неизвестные образцы				
Указан	Не учитывается	Не указан	Не указан	**Обнаружена ДНК Herpes simplex virus 2 (HSV2)**
Не указан	Не учитывается	**Указан**	Не указан	**Обнаружена ДНК Cytomegalovirus (CMV)**
Не указан	Не учитывается	Не указан	**Указан**	**Обнаружена ДНК Herpes simplex virus 1 (HSV1)**
Не указан	**Указан**	Не указан	Не указан	Не обнаружена ДНК выявляемых микроорганизмов
Не указан	Не указан	Не указан	Не указан	Недостоверный результат
Отрицательный контрольный образец				
Не указан	**Указан**	Не указан	Не указан	**Отрицательный результат** Результаты постановки валидны
Положительный контрольный образец				
Указан	**Указан**	**Указан**	**Указан**	**Положительный результат** Результаты постановки валидны

9.4 Недостоверный результат может быть связан с присутствием ингибиторов в препарате ДНК, полученном из биологического материала; неверным выполнением протокола анализа; несоблюдением температурного режима амплификации и др. В этом случае требуется повторное проведение ПЦР с имеющимся препаратом ДНК, либо повторное выделение ДНК и постановка ПЦР для этого образца, либо повторное взятие биологического материала у пациента (выполняется последовательно).

9.5 Если для биологического образца получены значения Cp/Cq/Ct менее 24 по каналам детекции Fam/Green, Rox/ Orange или Cy5/Red, то это говорит о высокой первоначальной концентрации ДНК соответствующего микроорганизма. В данном случае возможно получение ложноотрицательного результата для микроорганизма, ДНК которого присутствует в низкой концентрации. Для исключения ложноотрицательных результатов

рекомендуется повторно провести ПЦР выделенного препарата ДНК с использованием Набора реагентов для выявления ДНК вируса простого герпеса человека 1, 2 типов (HSV 1, 2) методом полимеразной цепной реакции (ВПГ-ГЕН), ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2008/03946 и Набора реагентов для выявления ДНК цитомегаловируса человека (CMV) методом полимеразной цепной реакции (ЦМВ-ГЕН), ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2008/03945.

9.6 При получении положительного результата для отрицательного контрольного образца результаты всей постановочной серии считают недостоверными. В этом случае необходимо проведение специальных мероприятий для выявления и устранения возможной контаминации.

9.7 При получении отрицательного результата для положительного контрольного образца результаты всей постановочной серии считают недостоверными. В этом случае требуется повторная постановка амплификации всей партии образцов.

10 ТРАНСПОРТИРОВАНИЕ, ХРАНЕНИЕ И ЭКСПЛУАТАЦИЯ

10.1 Транспортирование

10.1.1 Транспортирование набора реагентов осуществляют в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера, соответствующей условиям хранения компонентов, входящих в состав набора реагентов.

10.1.2 Фасовка S

Допускается транспортирование набора реагентов в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера от 2 °С до 25 °С не более 5 суток.

10.1.3 Фасовка U

10.1.3.1 Допускается транспортирование набора реагентов, за исключением полимеразы ТехноТаq MAX, в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера от 2 °С до 25 °С не более 5 суток.

10.1.3.2 Допускается транспортирование полимеразы ТехноТаq MAX в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера до 25 °С не более 5 суток.

10.1.4 Наборы реагентов, транспортированные с нарушением температурного режима, применению не подлежат.

10.2 Хранение

10.2.1 Фасовка S

Все компоненты набора реагентов следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов. Смесь для амплификации, запечатанную парафином, следует хранить в защищённом от света месте.

10.2.2 Фасовка U

10.2.2.1 Все компоненты набора реагентов, за исключением полимеразы ТехноТаq MAX, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов. Смесь для амплификации следует хранить в защищённом от света месте.

10.2.2.2 Полимеразу ТехноТаq MAX следует хранить в морозильной камере при температуре от минус 22 °С до минус 18 °С в течение всего срока годности набора реагентов.

10.2.3 Наборы реагентов, хранившиеся с нарушением регламентированного режима, применению не подлежат.

10.3 Указания по эксплуатации

10.3.1 Набор реагентов должен применяться согласно действующей версии утвержденной инструкции по применению.

10.3.2 Для получения достоверных результатов необходимо строгое соблюдение инструкции по применению набора реагентов.

10.3.3 После вскрытия упаковки компоненты набора реагентов следует хранить при следующих условиях:

- все компоненты набора реагентов, за исключением полимеразы ТехноТаq MAX, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов;
- смесь для амплификации и смесь для амплификации, запечатанную парафином, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в защищённом от света месте в течение всего срока годности набора реагентов.
- полимеразу ТехноТаq MAX (фасовка U) следует хранить в морозильной камере при температуре от минус 22 °С до минус 18 °С в течение всего срока годности набора реагентов.

10.3.4 Наборы реагентов с истекшим сроком годности применению не подлежат.

11 УКАЗАНИЯ ПО УТИЛИЗАЦИИ

11.1 При использовании набора реагентов в клинико-диагностической лаборатории образуются отходы класса В, которые утилизируются в соответствии с требованиями СанПиН 2.1.3684-21 и МУ 1.3.2569-09.

11.2 Наборы реагентов, пришедшие в непригодность, в том числе в связи с истечением срока годности, повреждением упаковки, подлежат утилизации в соответствии с требованиями СанПиН 2.1.3684-21.

12 ГАРАНТИИ ИЗГОТОВИТЕЛЯ

12.1 Предприятие-изготовитель гарантирует соответствие набора реагентов требованиям технических условий при соблюдении условий транспортирования, хранения и эксплуатации, установленных техническими условиями.

12.2 Срок годности набора реагентов – 12 месяцев при соблюдении всех условий транспортирования, хранения и эксплуатации.

13 РЕМОНТ И ТЕХНИЧЕСКОЕ ОБСЛУЖИВАНИЕ

Набор реагентов предназначен для однократного применения и не подлежит техническому обслуживанию и текущему ремонту.

14 СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ ПРИ МАРКИРОВКЕ НАБОРА РЕАГЕНТОВ

	Медицинское изделие для диагностики *in vitro*		Номер по каталогу
	Температурный диапазон		Изготовитель
	Содержимого достаточно для проведения <n> тестов		Не допускать воздействия солнечного света
	Использовать до		Нестерильно
	Код партии (серии)		Обратитесь к инструкции по применению или к инструкции по применению в электронном виде
	Дата изготовления		

15 ПЕРЕЧЕНЬ ПРИМЕНЯЕМЫХ НАЦИОНАЛЬНЫХ СТАНДАРТОВ

ГОСТ ISO 14971-2021 Изделия медицинские. Применение менеджмента риска к медицинским изделиям

ГОСТ 15.309-98 Система разработки и постановки продукции на производство. Испытания и приемка выпускаемой продукции. Основные положения

ГОСТ Р 2.105-2019 Единая система конструкторской документации (ЕСКД). Общие требования к текстовым документам

ГОСТ Р 15.013-2016 Система разработки и постановки продукции на производство (СРПП). Медицинские изделия

ГОСТ Р 51088-2013 Медицинские изделия для диагностики ин витро. Реагенты, наборы реагентов, тест-системы, контрольные материалы, питательные среды. Требования к изделиям и поддерживающей документации

ГОСТ Р 51352-2013 Медицинские изделия для диагностики ин витро. Методы испытаний

ГОСТ Р ИСО 15190-2023 Лаборатории медицинские. Требования безопасности

ГОСТ Р ИСО 15223-1-2023 Изделия медицинские. Символы, применяемые для передачи информации, предоставляемой изготовителем. Часть 1. Основные требования

ГОСТ Р ИСО 18113-1-2015 Медицинские изделия для диагностики in vitro. Информация, предоставляемая изготовителем (маркировка). Часть 1. Термины, определения и общие требования

ГОСТ Р ИСО 18113-2-2015 Медицинские изделия для диагностики in vitro. Информация, предоставляемая изготовителем (маркировка). Часть 2. Реагенты для диагностики in vitro для профессионального применения

ГОСТ Р ИСО 23640-2015 Изделия медицинские для диагностики in vitro. Оценка стабильности реагентов для диагностики in vitro

ГОСТ Р 53022.3-2008 Требования к качеству клинических лабораторных исследований, Ч.3. Правила оценки клинической информативности лабораторных тестов.

Примечание – Указанные выше стандарты были действующими на момент утверждения инструкции по применению. В дальнейшем, при пользовании документом, целесообразно проверить действие ссылочных нормативных документов на текущий момент. Если ссылочный документ заменён или изменён, то при применении настоящего документа следует пользоваться заменённым (изменённым) документом.

16 АДРЕС ДЛЯ ОБРАЩЕНИЯ

Производство наборов реагентов имеет сертифицированную систему менеджмента качества и соответствует требованиям стандарта систем менеджмента качества ISO 9001 в области разработки, производства и продажи IVD реагентов и приборов для молекулярно-генетической диагностики, и другого лабораторного применения и ISO 13485 в области разработки, производства и продажи IVD реагентов и приборов для медицинской молекулярно-генетической диагностики.

Производитель: Общество с ограниченной ответственностью «ДНК-Технология ТС» (ООО «ДНК-Технология ТС»), Россия.

Адрес производителя: 117246, Россия, г. Москва, проезд Научный, д. 20, строение 4.

Место производства:

- ООО «ДНК-Технология ТС», 117246, Россия, г. Москва, проезд Научный, д. 20, строение 4;
- ООО «НПО ДНК-Технология»,142281, Россия, Московская область, г. Протвино, ул. Железнодорожная, д. 3.

По вопросам, касающимся качества набора реагентов, следует обращаться в службу клиентской поддержки.

Служба клиентской поддержки:
8-800-200-75-15 (для России, звонок бесплатный),
+7 (495) 640-16-93 (для стран СНГ и зарубежья, звонок платный).
E-mail: hotline@dna-technology.ru
www.dna-technology.ru

ПРИЛОЖЕНИЕ А

Параметры теста, которые необходимо внести в программное обеспечение детектирующих амплификаторов «ДТпрайм», «ДТлайт» при использовании набора реагентов HSV1/HSV2/CMV ГерпесКомплекс в фасовке S

1) Количество пробирок в тесте – 1;
2) Объём реакционной смеси – 35 мкл;
3) В окне «Программа амплификации» ввести следующие параметры:

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	30	1		Цикл
	94	1	30			
2	94	0	30	5		Цикл
	64	0	15		√	
3	94	0	10	45		Цикл
	64	0	15		√	
4	94	0	5	1		Цикл
5	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

4) Внести следующие параметры каналов детекции:

Fam	Hex	Rox	Cy5	Cy5.5
Herpes simplex virus 2 (HSV2)	ВК	Cytomegalovirus (CMV)	Herpes simplex virus 1 (HSV1)	-

[1] - допускается хранение при температуре 10 °С

ПРИЛОЖЕНИЕ Б

Параметры теста, которые необходимо внести в программное обеспечение детектирующих амплификаторов «ДТпрайм», «ДТлайт» при использовании набора реагентов HSV1/HSV2/CMV ГерпесКомплекс в фасовке U

1) Количество пробирок в тесте – 1;
2) Объём реакционной смеси – 18 мкл;
3) В окне «Программа амплификации» ввести следующие параметры:

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	5	15		Цикл
	94	0	5			
2	94	5	00	1		Цикл
3	94	0	30	5		Цикл
	64	0	15		√	
4	94	0	10	45		Цикл
	64	0	15		√	
5	94	0	5	1		Цикл
6	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

4) Внести следующие параметры каналов детекции:

Fam	Hex	Rox	Cy5	Cy5.5
Herpes simplex virus 2 (HSV2)	ВК	Cytomegalovirus (CMV)	Herpes simplex virus 1 (HSV1)	-

[1] - допускается хранение при температуре 10 °С

ДНК-Технология
117587, Россия, г. Москва,
вн. тер. г. муниципальный округ Чертаново Северное,
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8-800-200-75-15 (для России, звонок бесплатный)
+7 (495) 640-16-93 (для стран СНГ и зарубежья, звонок платный)
E-mail: hotline@dna-technology.ru

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ В СФЕРЕ ЗДРАВООХРАНЕНИЯ
(РОСЗДРАВНАДЗОР)

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ НА МЕДИЦИНСКОЕ ИЗДЕЛИЕ

от 07 февраля 2025 года № РЗН 2025/24371

На медицинское изделие
Набор реагентов для выявления ДНК Herpes Simplex Virus 1, Herpes Simplex Virus 2, Cytomegalovirus методом ПЦР в режиме реального времени (HSV1/HSV2/CMV ГерпесКомплекс)

Настоящее регистрационное удостоверение выдано
Общество с ограниченной ответственностью "ДНК-Технология ТС" (ООО "ДНК-Технология ТС"), Россия, 117246, Москва, Научный пр-д, д. 20, стр. 4

Производитель
Общество с ограниченной ответственностью "ДНК-Технология ТС" (ООО "ДНК-Технология ТС"), Россия, 117246, Москва, Научный пр-д, д. 20, стр. 4

Место производства медицинского изделия
см.приложение

Номер регистрационного досье **№ РД-66198/104541 от 24.12.2024**

Класс потенциального риска применения медицинского изделия **2б**

Код Общероссийского классификатора продукции по видам экономической деятельности **20.59.52.195**

Настоящее регистрационное удостоверение имеет приложение на 1 листе

приказом Росздравнадзора от 07 февраля 2025 года № 621
допущено к обращению на территории Российской Федерации.

Врио руководителя Федеральной службы по надзору в сфере здравоохранения **Д.В. Пархоменко**

0083885

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ В СФЕРЕ ЗДРАВООХРАНЕНИЯ
(РОСЗДРАВНАДЗОР)

ПРИЛОЖЕНИЕ
К РЕГИСТРАЦИОННОМУ УДОСТОВЕРЕНИЮ
НА МЕДИЦИНСКОЕ ИЗДЕЛИЕ

от 07 февраля 2025 года № РЗН 2025/24371

Лист 1

На медицинское изделие
Набор реагентов для выявления ДНК Herpes Simplex Virus 1, Herpes Simplex Virus 2, Cytomegalovirus методом ПЦР в режиме реального времени (HSV1/HSV2/CMV ГерпесКомплекс), в вариантах исполнения:
I. Фасовка S, стрипы:
- смесь для амплификации, запечатанная парафином - 12 стрипов по 8 пробирок (по 20 мкл);
- раствор Taq-полимеразы - 2 пробирки (по 500 мкл);
- минеральное масло - 2 пробирки (по 1,0 мл);
- положительный контрольный образец - 1 пробирка (130 мкл);
- крышки для стрипов - 12 шт.

II. Фасовка S, пробирки:
- смесь для амплификации, запечатанная парафином - 96 пробирок (по 20 мкл);
- раствор Taq-полимеразы - 2 пробирки (по 500 мкл);
- минеральное масло - 2 пробирки (по 1,0 мл);
- положительный контрольный образец - 1 пробирка (130 мкл).

III. Фасовка U:
- смесь для амплификации - 1 пробирка (600 мкл);
- полимераза ТехноТаq MAX - 1 пробирка (30 мкл);
- ПЦР-буфер - 1 пробирка (600 мкл);
- положительный контрольный образец - 1 пробирка (130 мкл).

Комплектность:
- набор реагентов в одном из вариантов исполнения - 1 шт.;
- вкладыш - 1 экз.;
- инструкция по применению - 1 экз.;
- паспорт - 1 экз.

Место производства:
1. ООО "ДНК-Технология ТС", Россия, 117246, Москва, Научный пр-д, д. 20, стр. 4.
2. ООО "НПО ДНК-Технология", Россия, 142281, Московская область, г. Протвино, ул. Железнодорожная, д. 3.

Врио руководителя Федеральной службы по надзору в сфере здравоохранения **Д.В. Пархоменко**

0154426

DNA-TECHNOLOGY

831-2 2024-04-22

CE IVD

For professional use only

HHV6 REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF

R1-P202-S3/9EU
R1-P202-23/9EU
R1-P202-UA/9EU

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1. INTENDED USE

The **HHV6 REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HHV6 REAL-TIME PCR Detection Kit** is designed to detect HHV6 nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: blood, liquor, bioptate or punctate from foci of organ and tissue lesions, oropharyngeal smears, saliva.

Indications: symptoms of infection caused by HHV6, monitoring the effectiveness of antiviral treatment, differential diagnosis of infections with similar clinical manifestations, in the complex examination of patients with lymphoproliferative diseases and hematoblastosis, examination of organ and tissue recipients before and after transplantation.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HHV6 REAL-TIME PCR Detection Kit.**

The **HHV6 REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

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 the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only 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.

The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the HHV6 product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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
HHV6	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **HHV6 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 **HHV6 REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **HHV6 REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **HHV6 REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P202-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white 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		

* - marking as C+ is allowed

Table 3. The **HHV6 REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P202-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white 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

* - marking as C+ is allowed

Table 4. The **HHV6 REAL-TIME PCR Detection Kit** content, package U for R1-P202-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or slightly pink transparent liquid	600 µL	1tube
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 (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) or on using a dosing device it is possible to run 96 tests simultaneously (94 experimental samples, negative control and positive control samples).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, sterile single use containers to collect clinical material;
- Sterile tubes containing transport media: “DNA-Technology” **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU) or equivalent for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- High speed centrifuge (RCF(g) no less than 16000) for 1.5 mL tubes;
- High speed centrifuge (RCF(g) no less than 1150) for 4.5 mL tubes;
- Solid-state thermostat (temperature range 25-98 °C);
- Vortex mixer;
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU) (not applicable to male urethral swabs) and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- 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 (using detection kit in the package U REF R1-P202-UA/9EU);
- Vortex mixer;
- Vortex rotor for strips (using detection kit package S, strips REF R1-P202-S3/4EU);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes or strips;
- 0.2 mL PCR tubes (using detection kit in the package U REF R1-P202-UA/9EU);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- DTstream M1 dosage instrument (only for automated dosing using detection kit in the package U REF R1-P202-UA/9EU);
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing using detection kit in the package U REF R1-P202-UA/9EU);
- Centrifuge for microtrays (only for automated dosing using detection kit in the package U REF R1-P202-UA/9EU);
- Polymer thermal seal for microtray sealing (only for automated dosing using detection kit in the package U REF R-P202-UA/9EU);
- PCR microtray (only for automated dosing using detection kit in the package U REF R1-P202-UA/9EU);
- 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 **HHV6 REAL-TIME PCR Detection Kit** except TechnoTaq MAX polymerase (package U) must be stored at temperatures from 2 °C to 8 °C over the storage period. TechnoTaq MAX polymerase must be stored at temperatures from minus 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 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 **HHV6 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 **HHV6 REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **HHV6 REAL-TIME PCR Detection Kit**.

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 **HHV6 REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, liquor, bioptate or punctate from foci of organ and tissue lesions, oropharyngeal smears, saliva.

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.

PCR inhibitors are the presence of hemoglobin in a DNA sample as a result of incomplete removal during DNA extraction from biomaterial sample containing blood impurities, as well as the presence of isopropyl alcohol and methyl acetate in a DNA sample as a result of incomplete removal of washing solutions during sample preparation.

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

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

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.

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.

Peripheral blood

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. The use of sodium citrate anticoagulant is also applicable. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

ATTENTION! It is not allowed to use heparin as an anticoagulant.

Liquor, saliva, punctate

Collect liquor, saliva punctate (approximately 500 µL) into the sterile container and close it tightly.

Bioptates

Bioptates are transferred into a 1.5 mL tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR. After sample collection the tube is tightly closed and marked.

Oropharyngeal smears

Sampling is carried out using sterile disposable swabs or tampons according to the established procedure.

Take the smear with a dry sterile disposable swab into tubes with a transport medium designed by manufacturer for transporting and storage of biomaterial for PCR assays.

Order of taking:

1 Take the smear with a swab with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.

2 Open the tube.

3 Put the swab into the tube with transport medium, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.

4 Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.

5 Close the tube tightly and mark it.

Method limitations – topical use of medications less than 24 hours before the assay.

Transportation and storage of the samples

Samples may be transported and stored 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 18 °C to minus 22 °C for one month.

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

Sample preparation

Peripheral blood

The detailed description of peripheral blood preprocessing procedure is cited in extraction kits user manuals.

Liquor, saliva, punctate

1. Transfer 500 µL of biomaterial into 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperature (from 18 °C to 25 °C).
3. Remove the supernatant, leaving approximately 50 µL in the tube (precipitate + liquid fraction).
4. Add 500 µL of sterile physiological solution to the precipitate.
5. Vortex the tube for 3-5 seconds, then spin down drops for 3-5 seconds.
6. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
7. Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Bioptates

1. Vortex the tube with sample for 3-5 seconds, then spin down drops for 3-5 seconds.
2. Remove supernatant.

The following sample preparation is made according to user manual for the extraction kit used.

ATTENTION! When extracting DNA from biopsy specimens, only **PREP-NA** and **PREP-GS** extraction kits should be used.

Oropharyngeal smears

1 Centrifuge the tube with the sample at RCF(g) 16000 for 10 minutes at room temperature (from 18 °C to 25 °C).

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.

ATTENTION! The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements are cited in **PREP-NA**, **PREP-GS, PREP-RAPID** and **PREP-MB RAPID** extraction kits user manuals.

No additional pretreatment is required in case of taking smears into tubes with the **PREP-RAPID** reagent.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. For DNA extraction from peripheral blood, it is allowed to use any reagent kits registered as a medical device and recommended by the manufacturers for DNA extraction 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 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-P202-S3/9EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips from the other kits!

8.1.1. Mark tubes with 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 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 (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes.

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 in 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 in 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-" and "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 (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

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.

For use with Rotor-Gene Q real-time thermal cycler consult user manual for devices. See Table 6.

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							

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 2	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement.			

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

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

ATTENTION! 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 in vortex for 1-3 seconds to collect the drops.

ATTENTION! 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 with PCR-mix.

ATTENTION! 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 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 in 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 in 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 (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

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 7.

Amplification program for Rotor-Gene Q thermocycler is contained in the Table 8.

Table 7. The PCR program for DTlite and DTprime Thermal Cyclers for package U

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	10[1]			Holding		Holding
[1] – holding at 25°C is allowed						

Table 8. The PCR program for Rotor-Gene Q thermal cycler for package U

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 2	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

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

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

8.3 Preparing PCR using DTStream (only for package U)

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 in vortex for 1-3 seconds to collect the drops.

ATTENTION! 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, the spin in vortex for 1-3 seconds to collect the drops.

8.3.5 Vortex the tubes with DNA samples, “C-” and “C+” for 3-5 seconds and spin down the drops in vortex 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 in a vortex mixer.

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 on RCF(g) 100 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 7.

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

9. CONTROLS

The **HHV6 REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the HHV6 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.

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 in volumes as instructions.

For **HHV6 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 **HHV6 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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing HHV6 DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of HHV6 DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

11. SPECIFICATIONS

a. The analytical **specificity** of the **HHV6 REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with HHV6 DNA are to be registered positive for specific product (a fragment of the HHV6 genome). The samples free of HHV6 DNA are to be registered negative for specific product and positive for internal control.

No nonspecific positive amplification results were shown in the presence of *Cytomegalovirus*, *Herpes symplex virus 1,*2, *Epstein-Barr virus*, *Human herpes virus 8*, *Varicella-Zoster virus*, *HPV 6*, *HPV 11* DNA, and human DNA at a concentration of up to 1.0×10^8 copies/mL of the sample.

b. The analytical **sensitivity** is 5 copies of Human Herpes virus type 6 DNA per amplification tube.

The analytical sensitivity was set by analyzing serial dilutions of a laboratory control (LC). A total of 94 runs were made for each concentration.

LC concentration, copies per amplification tube	Number of runs	Number of positive results	% of positive results
5	94	93	98.9
2	94	89	94.6
0.5	94	60	63.8
0	94	0	0

Note. The analytical sensitivity for DNA in the sample depends on the sample preparation method and the final volume of extracted DNA (elution volume).

E x a m p l e : The analytical sensitivity of 5 DNA copies per amplification tube corresponds to the following values of DNA concentration when using nucleic acid extraction kits manufactured by "DNA-Technology R&P", LLC:

Sample	**Nucleic acids extraction kits**			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution of 300 µL)**	**PREP-RAPID**
- oropharyngeal mucosa smear in 500 µL of transport medium; - punctate (when extracted from 500 µL sample); - bioptates*	50 copies/ sample	100 copies/ sample	300 copies/sample	500 copies/sample
* Only **PREP-GS** and **PREP-NA** extraction kits are used for DNA extraction from bioptates				

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU, **PREP-RAPID** REF P-001/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits.

12. TROUBLESHOOTING

Table 9. 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

"DNA-Technology Research&Production", LLC declares that the abovementioned 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 official representative in EU by quality issues of **HHV6 REAL-TIME PCR Detection Kit** Technical support.

Technical support:

E-mail: hotline@dna-technology.ru

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14. KEY TO 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
VER	Version	CONTROL +	Positive control
NON STERILE	Non-sterile		Caution
EC REP	Authorized representative in the European Community		

REF	R1-P202-S3/9EU R1-P202-23/9EU R1-P202-UA/9EU
VER	831-2.2024.04.22

DNA-TECHNOLOGY

417-4 2024-04-22

CE IVD

For professional use only

Epstein Barr virus REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF R1-P205-S3/9EU

R1-P205-23/9EU

VER 417-4.2024.04.22

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1. INTENDED USE

The **Epstein Barr virus REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Epstein Barr virus REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Epstein Barr virus REAL-TIME PCR Detection Kit** is designed to detect Epstein Barr virus nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: blood, synovial fluid, cerebrospinal fluid, amniotic fluid, biopsy material or punctate from lesions of organs and tissues, scrapes from the oropharynx, saliva.

Indications for the use: symptoms of infection caused by EBV (infectious mononucleosis), monitoring the effectiveness of antiviral treatment, differential diagnosis of infections with similar clinical implications, examination of recipients of organs and tissues before and after transplantation.

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

The **Epstein Barr virus REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by paraffin layer. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **Epstein Barr virus REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the Epstein Barr virus product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the 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
Epstein Barr virus	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers or Bio-Rad made iCycler iQ or iQ5 or QIAGEN made Rotor-Gene Q for **Epstein Barr virus 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 **Epstein Barr virus REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Epstein Barr virus REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P205-S3/9EU and R1-P205-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL per tube)	96 tubes or 12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL per tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL per tube)	2 tubes
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps [1]	12 8-caps		

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

The **Epstein Barr virus REAL-TIME PCR Detection Kit** is intended for single use and designed for 96 tests (no more than 94 defined samples, one positive control and one negative control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use sterile containers to collect clinical material;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or physiological saline solution or sterile PBS for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

  Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

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 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);

[1] - for detection kit packaged in strips REF R1-P205-S3/9EU

- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID** (REF P-001/1EU), **PREP-NA** REF P-002/1EU), **PREP-GS** (REF P-003/1EU) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (using detection kit packaged in strips REF R1-P205-S3/9EU);
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Epstein Barr virus REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °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 excessive temperature and light can be detrimental to product performance.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °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 **Epstein Barr virus 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 **Epstein Barr virus REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Epstein Barr virus REAL-TIME PCR Detection Kit.**

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 **Epstein Barr virus REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, synovial fluid, cerebrospinal fluid, amniotic fluid, biopsy material or punctate from lesions of organs and tissues, scrapes from the oropharynx, saliva, depending on professional prescription.

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

General requirements

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results. PCR research is a direct method, so taking of biological material must be carried out from the location of the infectious process.

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.

PCR inhibitors are the presence of hemoglobin in the DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

Impurities contained in the biomaterial sample are almost completely removed during the DNA extraction. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible

Sample collection

Blood sampling

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

Synovial fluid, cerebrospinal fluid, amniotic fluid, saliva, punctate

Synovial fluid, cerebrospinal fluid, amniotic fluid, saliva, punctuate (about 500 µL) are collected in a sterile container, closed tightly and marked.

Bioptates sampling

Bioptates are transferred to a 1.5 mL tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR. After sample collection the tube is tightly closed and marked.

Scrapes from the oropharynx sampling

Take the scrape with a dry sterile disposable swab into 1.5 mL plastic tubes with 300 µL of sterile saline solution or a transport medium.

Order of taking:

1. Take the scrape with a swab with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.
2. Open the tube.

3. Put the swab into the tube with transport medium, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the swab from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used swab.
5. Close the tube tightly and mark it.

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

Transportation and storage of the samples

Samples may be transported and stored in physiological saline solution 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 18 °C to minus 22 °C for one month.

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

Sample preparation

Synovial fluid, cerebrospinal fluid, amniotic fluid, saliva, punctate

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

1. Transfer 500 µL of the material into a 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperatures (from 18 °C to 25 °C).
3. Remove the supernatant, leaving 50 µL in tube (precipitate + liquid fraction).
4. Add 500 µL of a sterile saline solution to the precipitate.
5. Vortex the tube for 3-5 seconds, then spin for 3-5 seconds.
6. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
7. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS**. Tightly close the tubes.

The resulting material is ready for DNA extraction.

Biopsy samples

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

1. Vortex the tubes with samples for 3-5 seconds and spin down drops for 3-5 seconds.
2. Remove the supernatant.

The resulting material is ready for DNA extraction.

Scrapes from the oropharynx

1. Centrifuge the tube containing the sample at RCF(g) 16000 for 10 minutes at room temperature (from 18 °C to 25 °C).
2. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS.** Tightly close the tubes.

The resulting material is ready for DNA extraction.

When taking scrapes into tubes with the “PREP-RAPID” reagent, sample preparation is not required, the material is ready for DNA extracting.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID** 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.

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 in volumes as indicated.

Assay procedure

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

When using package S, 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 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes.

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

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 in a vortex mixer.

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

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 before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

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

8.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-” tube and positive control (C+) into corresponding tube. Avoid paraffin layer break.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

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

8.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], 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 8.9) and run PCR. See Tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

Amplification products can be stored at temperatures from 2 °C to 8 °C for one month or at temperatures from minus 20 °C for 12 months.

Table 3. 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						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4				10	Storage

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

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5				10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	

* Take the measurement.

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Epstein Barr virus REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the Epstein Barr virus 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.

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

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.

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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing Epstein Barr virus DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of Epstein Barr virus DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Epstein Barr virus REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with Epstein Barr virus DNA are to be registered positive for specific product (a fragment of the Epstein Barr virus genome). The samples free of Epstein Barr virus DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of Cytomegalovirus, Herpes symplex virus 1,2, Human herpesvirus 6, Human herpesvirus 8, Varicella-Zoster virus, HPV 6, HPV 11, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. In a determination of analytical sensitivity, the **Epstein Barr virus REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of EBV 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
5.0	94	94	100
2.0	94	89	94.6
0.5	94	60	63.8
0.0	94	0	0.0

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

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of EBV in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID** (at elution in 300 µL)	**PREP-RAPID**
- scrape from the oropharynx in 500 µL transport medium; - synovial fluid, amniotic fluid, punctate (extracting from 500 µL of sample); - biopsy material	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** REF P-001/1EU, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits.

12. TROUBLESHOOTING

Table 8. 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

"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 official representative in EU by quality issues of **Epstein Barr virus REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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14. KEY TO SYMBOLS

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
	Caution	VER	Version
	Do not reuse	CONTROL +	Positive control
EC REP	Authorized representative in the European Community	NON STERILE	Non-sterile

REF R1-P205-S3/9EU

R1-P205-23/9EU

VER 417-4.2024.04.22

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ В СФЕРЕ ЗДРАВООХРАНЕНИЯ
(РОСЗДРАВНАДЗОР)

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ НА МЕДИЦИНСКОЕ ИЗДЕЛИЕ

от 16 декабря 2024 года № РЗН 2024/24242

На медицинское изделие
Набор реагентов для выявления ДНК *Toxoplasma gondii* методом ПЦР в режиме реального времени (Toxoplasms gondii)

Настоящее регистрационное удостоверение выдано
**Общество с ограниченной ответственностью "ДНК-Технология ТС" (ООО "ДНК-Технология ТС"), Россия,
117246, Москва, Научный пр-д, д. 20, стр. 4**

Производитель
**Общество с ограниченной ответственностью "ДНК-Технология ТС" (ООО "ДНК-Технология ТС"), Россия,
117246, Москва, Научный пр-д, д. 20, стр. 4**

Место производства медицинского изделия
см.приложение

Номер регистрационного досье **№ РД-64443/72797 от 16.09.2024**

Класс потенциального риска применения медицинского изделия **2б**

Код Общероссийского классификатора продукции по видам экономической деятельности **20.59.52.195**

Настоящее регистрационное удостоверение имеет приложение на 1 листе

приказом Росздравнадзора от 16 декабря 2024 года № 7123
допущено к обращению на территории Российской Федерации.

**Руководитель Федеральной службы
по надзору в сфере здравоохранения** **А.В. Самойлова**

0080955

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ В СФЕРЕ ЗДРАВООХРАНЕНИЯ
(РОСЗДРАВНАДЗОР)

ПРИЛОЖЕНИЕ
К РЕГИСТРАЦИОННОМУ УДОСТОВЕРЕНИЮ
НА МЕДИЦИНСКОЕ ИЗДЕЛИЕ

от 16 декабря 2024 года № РЗН 2024/24242

На медицинское изделие
Набор реагентов для выявления ДНК *Toxoplasma gondii* методом ПЦР в режиме реального времени (Toxoplasms gondii) , в вариантах исполнения:
I. Фасовка S, стрипы, в составе:
- смесь для амплификации, запечатанная парафином - 12 стрипов по 8 пробирок (по 20 мкл);
- раствор Taq-полимеразы - 2 пробирки (по 500 мкл);
- минеральное масло - 2 пробирки (по 1,0 мл);
- положительный контрольный образец - 1 пробирка (130 мкл);
- крышки для стрипов - 12 шт.
- вкладыш - 1 экз.;
- инструкция по применению - 1 экз.;
- паспорт - 1 экз.

II. Фасовка S, пробирки, в составе:
- смесь для амплификации, запечатанная парафином - 96 пробирок (по 20 мкл);
- раствор Taq-полимеразы - 2 пробирки (по 500 мкл);
- минеральное масло - 2 пробирки (по 1,0 мл);
- положительный контрольный образец - 1 пробирка (130 мкл);
- вкладыш - 1 экз.;
- инструкция по применению - 1 экз.;
- паспорт - 1 экз.

III. Фасовка U, в составе:
- смесь для амплификации - 1 пробирка (600 мкл);
- полимераза ТехноТаq MAX - 1 пробирка (30 мкл);
- ПЦР-буфер - 1 пробирка (600 мкл);
- положительный контрольный образец - 1 пробирка (130 мкл);
- вкладыш - 1 экз.;
- инструкция по применению - 1 экз.;
- паспорт - 1 экз.

Место производства:
1. ООО "ДНК-Технология ТС", Россия, 117246, Москва, Научный пр-д, д. 20, стр. 4.
2. ООО "НПО ДНК-Технология", Россия, 142281, Московская область, г. Протвино, ул. Железнодорожная, д. 3.

Руководитель Федеральной службы по надзору в сфере здравоохранения **А.В. Самойлова**

0155325

ДНК-ТЕХНОЛОГИЯ

453 2024-12-19

IVD

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления ДНК *Toxoplasma gondii*
методом ПЦР в режиме реального времени

Toxoplasma gondii

Регистрационное удостоверение
№ РЗН 2024/24242 от 16 декабря 2024 года

ВНИМАНИЕ! Изучите инструкцию перед началом работы

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СПИСОК СОКРАЩЕНИЙ И ОБОЗНАЧЕНИЙ

В настоящей инструкции используются следующие сокращения и обозначения:

RCF	- от англ. relative centrifugal force, относительное ускорение центрифуги
ВК	- внутренний контроль
ДНК	- дезоксирибонуклеиновая кислота
ДНКазы	- дезоксирибонуклеазы
К-	- отрицательный контрольный образец
К+	- положительный контрольный образец
ЛКО	- лабораторный контрольный образец
НК	- нуклеиновые кислоты (РНК и ДНК)
ПЦР	- полимеразная цепная реакция
РНКазы	- рибонуклеазы

1 ПРЕДНАЗНАЧЕННОЕ ПРИМЕНЕНИЕ

1.1 Полное наименование набора реагентов: Набор реагентов для выявления ДНК *Toxoplasma gondii* методом ПЦР в режиме реального времени (Toxoplasma gondii), далее по тексту – набор реагентов.

1.2 Назначение: набор реагентов предназначен для выявления ДНК *Toxoplasma gondii* в биологическом материале человека (кровь, спинномозговая жидкость, амниотическая жидкость, биоптат или пунктат из очагов поражения органов и тканей) методом ПЦР в режиме реального времени.

1.3 Функциональное назначение: диагностика *in vitro*.

1.4 Показания к проведению исследования: симптомы инфекционного или воспалительного заболевания, вызванного *Toxoplasma gondii*.
Противопоказаний к применению нет.

1.5 Популяционные и демографические аспекты: применение набора реагентов не зависит от популяционных и демографических аспектов.

1.6 Область применения: набор реагентов может быть использован в клинико-диагностических лабораториях медицинских учреждений.

1.7 Потенциальные пользователи: квалифицированный персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории: врач клинико-диагностической лаборатории, фельдшер-лаборант (медицинский лабораторный техник).

1.8 Применять набор реагентов строго по назначению согласно данной инструкции по применению.

2 ХАРАКТЕРИСТИКА НАБОРА РЕАГЕНТОВ

2.1 Состав набора реагентов

REF R1-P031-S3/9, фасовка S, стрипы			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации, запечатанная парафином	Прозрачная бесцветная или розовая жидкость под воскообразным белым слоем	12 стрипов по 8 пробирок	по 20 мкл
Раствор Taq-полимеразы	Прозрачная бесцветная жидкость	2 пробирки	по 500 мкл
Минеральное масло	Прозрачная бесцветная вязкая маслянистая жидкость	2 пробирки	по 1,0 мл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл
Крышки для стрипов	12 шт.		

REF R1-P031-23/9, фасовка S, пробирки			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации, запечатанная парафином	Прозрачная бесцветная или розовая жидкость под воскообразным белым слоем	96 пробирок	по 20 мкл
Раствор Taq-полимеразы	Прозрачная бесцветная жидкость	2 пробирки	по 500 мкл
Минеральное масло	Прозрачная бесцветная вязкая маслянистая жидкость	2 пробирки	по 1,0 мл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл

REF R1-P031-UA/9, фасовка U			
Наименование компонента	Внешний вид	Количество пробирок	Номинальный объём компонента
Смесь для амплификации	Прозрачная бесцветная или розовая жидкость	1 пробирка	600 мкл
Полимераза ТехноTaq MAX	Прозрачная бесцветная вязкая жидкость	1 пробирка	30 мкл
ПЦР-буфер	Прозрачная бесцветная жидкость	1 пробирка	600 мкл
Положительный контрольный образец[1]	Прозрачная бесцветная жидкость	1 пробирка	130 мкл

Все компоненты набора реагентов готовы к применению и не требуют дополнительной подготовки к работе.

Комплектность:

- Набор реагентов в одном из вариантов исполнения – 1 шт.
- Инструкция по применению – 1 экз.
- Вкладыш – 1 экз.
- Паспорт – 1 экз.

[1] - на этикетке компонента для всех фасовок «Положительный контрольный образец» указывается как «К+»

2.2 Количество анализируемых образцов

Набор реагентов в фасовке S рассчитан на проведение 96 определений (не более 24 постановок), включая анализ неизвестных образцов, отрицательных контрольных образцов и положительных контрольных образцов.

Набор реагентов в фасовке U рассчитан на проведение 96 определений при условии постановки не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

2.3 Принцип метода

Метод: Полимеразная цепная реакция (ПЦР) с детекцией результатов в режиме реального времени; качественный анализ.

Принцип метода основан на использовании процесса амплификации ДНК с помощью полимеразной цепной реакции (ПЦР). Процесс амплификации заключается в серии повторяющихся циклов температурной денатурации ДНК, отжига праймеров с комплементарными последовательностями и последующей достройки полинуклеотидных цепей с этих праймеров Taq-полимеразой.

Для повышения чувствительности и специфичности реакции предусмотрено применение «горячего» старта, который обеспечивается для фасовки S методикой приготовления реакционной смеси, состоящей из двух слоёв, разделённых прослойкой из парафина. Смешение слоёв и превращение их в реакционную смесь происходит только после плавления парафина, что исключает неспецифический отжиг праймеров на ДНК-мишени при начальном прогреве пробирки. «Горячий» старт для фасовки U обеспечивается использованием полимеразы, активность которой блокирована антителами, активация фермента происходит только после предварительного прогрева реакционной смеси при 94 °С. Это исключает неспецифический отжиг праймеров на ДНК-мишени при начальном прогреве пробирки.

В реакционную смесь для амплификации введены ДНК-зонды, каждый из которых несёт флуоресцентную метку и гаситель флуоресценции. При образовании специфичного продукта ДНК-зонд разрушается, действие гасителя на флуоресцентную метку прекращается, что ведёт к возрастанию уровня флуоресценции, который фиксируется детектирующим амплификатором.

Количество разрушенных зондов (а, следовательно, и уровень флуоресценции) увеличивается пропорционально количеству образовавшихся специфических ампликонов. Уровень флуоресценции измеряется на каждом цикле амплификации в режиме реального времени.

В состав смеси для амплификации включен внутренний контроль (ВК), который предназначен для контроля прохождения полимеразной цепной реакции.

В состав ДНК-зондов, использующихся для детекции продукта амплификации искомой ДНК, включена флуоресцентная метка Fam. В состав ДНК-зондов, использующихся для детекции продукта амплификации внутреннего контроля, входит флуоресцентный краситель Hex. В таблице 1 приведены каналы детекции продуктов амплификации.

Таблица 1 – Каналы детекции продуктов амплификации

Fam/Green	Hex/Yellow/Vic	Rox/Orange	Cy5/Red	Cy5.5/Crimson
Toxoplasma gondii	ВК	-	-	-

Исследование состоит из следующих этапов: выделение ДНК (пробоподготовка), ПЦР-амплификация ДНК с одновременной детекцией результатов с использованием набора реагентов Toxoplasma gondii.

2.4 Время проведения анализа (включая пробоподготовку): от 2 часов (в зависимости от количества образцов и используемого набора/комплекта реагентов для выделения ДНК).

3 АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1 Аналитическая специфичность

В образцах биологического материала человека, содержащих ДНК *Toxoplasma gondii*, программное обеспечение детектирующего амплификатора должно регистрировать положительный результат амплификации специфического продукта (фрагмента генома *Toxoplasma gondii*) по каналу детекции Fam/Green.

В образцах биологического материала, не содержащих ДНК *Toxoplasma gondii*, программное обеспечение детектирующего амплификатора должно регистрировать отрицательный результат амплификации специфического продукта (фрагмента генома *Toxoplasma gondii*) и положительный результат амплификации внутреннего контроля по каналу детекции Hex/Yellow/Vic.

Показано отсутствие неспецифических положительных результатов амплификации при наличии в образце ДНК следующих микроорганизмов*: Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Ureaplasma parvum*, *Neisseria gonorrhoeae*, *Candida albicans*, а также ДНК человека в концентрации до $1{,}0\times10^8$ копий/мл образца.

3.2 Интерферирующие вещества

Наличие ингибиторов ПЦР в образце биологического материала может быть причиной сомнительных (неопределённых/недостоверных) результатов. Признаком ингибирования ПЦР является одновременное отсутствие амплификации внутреннего контроля и специфического продукта (см.2.3, 9.3, 9.4).

К ингибиторам ПЦР отнесены следующие вещества: гемоглобин и лекарственные препараты, присутствующие в образце ДНК в результате неполного удаления в процессе выделения ДНК из образца биоматериала, а также изопропиловый спирт и метилацетат, присутствующие в образце ДНК в результате неполного удаления промывочных растворов в ходе пробоподготовки.

Максимальные концентрации интерферирующих веществ, при которых не наблюдалось влияние на амплификацию лабораторных контрольных образцов и внутреннего контрольного образца составляют: гемоглобин – 0,35 мг/мл образца ДНК, изопропиловый спирт – 100 мкл/мл образца ДНК, метилацетат – 100 мкл/мл образца ДНК.

Для оценки возможной интерференции лекарственных препаратов были выбраны те, которые потенциально могут присутствовать в остаточных количествах в биологических образцах человека, взятых из соответствующих исследуемых биотопов (Мирамистин®, хлоргексидин биглюконат).

Для всех исследуемых лекарственных препаратов было показано отсутствие их влияния в концентрации до 10% в образце биоматериала.

3.3 Предел обнаружения

5 копий ДНК *Toxoplasma gondii* на амплификационную пробирку.

Предел обнаружения установлен путем анализа серийных разведений лабораторного контрольного образца (ЛКО).

Предел обнаружения соответствует следующим значениям концентрации ДНК при использовании указанных наборов/комплектов реагентов для выделения ДНК и конечного объёма элюции (разведения) выделенной ДНК:

Биоматериал	**Наименование комплекта/набора для выделения ДНК**	**Объём полученного препарата, мкл**	**Предел обнаружения, копий/образец**
Спинномозговая жидкость (при выделении из 500 мкл образца)	ПРОБА-НК	50	50
	ПРОБА-ГС	100	100
	ПРОБА-МЧ-РАПИД	100	100
	ПРОБА-РАПИД	500	500
Амниотическая жидкость (при выделении из 500 мкл образца)	ПРОБА-МЧ-РАПИД	100	100
Спинномозговая жидкость, амниотическая жидкость (при выделении из 1,0 мл образца)	ПРОБА-ОПТИМА	400	400
Биоптаты/пунктаты	ПРОБА-НК	50	50
	ПРОБА-ГС	100	100
	ПРОБА-ОПТИМА	400	400
Цельная периферическая кровь (500 мкл[1])	ПРОБА-ОПТИМА МАКС	100	100
Цельная периферическая кровь (100 мкл)	ПРОБА-МЧ-МАКС	50	50

3.4 Диагностические характеристики

Вид биоматериала	**Диагностическая чувствительность**		**Диагностическая специфичность**	
	Исследовано образцов	Значение (95 % ДИ)	Исследовано образцов	Значение (95 % ДИ)
Кровь	25	100 % (86,28 % – 100 %)	25	100 % (86,28 % – 100 %)
Биоптаты, пунктаты из очагов поражения органов и тканей	30	100 % (88,43 % – 100 %)	50	100 % (92,89 % – 100 %)
Спинномозговая жидкость	25	100 % (86,28 % – 100 %)	25	100 % (86,28 % – 100 %)
Амниотическая жидкость	25	100 % (86,28 % – 100 %)	25	100 % (86,28 % – 100 %)
Итого	105	100 % (96,55 % – 100 %)	125	100 % (97,09 % – 100 %)

3.5 Воспроизводимость и повторяемость

Воспроизводимость составляет 100 %

Повторяемость составляет 100 %.

[1] - при добавлении 100 мкл лизирующего раствора

4 МЕРЫ ПРЕДОСТОРОЖНОСТИ

Организация работы ПЦР-лаборатории, оборудование и материалы должны соответствовать требованиям ГОСТ Р ИСО 15190-2023, методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот, при работе с материалом, содержащим микроорганизмы I-IV групп патогенности», с соблюдением санитарных правил и норм СанПиН 3.3686-21 «Санитарно-эпидемиологические требования по профилактике инфекционных болезней».

Неизвестные образцы рассматриваются как потенциально-опасные. При работе с набором реагентов следует надевать одноразовые перчатки без талька.

При работе с микроорганизмами I-IV групп патогенности выбор типа защитного костюма (рабочей одежды и средств индивидуальной защиты) проводится в строгом соответствии с санитарными правилами и нормами СанПиН 3.3686-21 и определяется видом возбудителя, рабочей зоной, оснащением ее боксами биологической безопасности.

Следует использовать только одноразовые наконечники и пробирки.

Не допускается использование одних и тех же наконечников при обработке различных образцов биологического материала.

К работе с набором реагентов допускается персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории.

Выделение ДНК следует проводить в боксах биологической безопасности II класса. Подготовку к ПЦР с использованием набора реагентов возможно проводить в ПЦР-боксах.

Запрещается перемещение лабораторного оборудования, в том числе дозаторов, штативов, лабораторной посуды, халатов, головных уборов и пр., а также растворов реагентов из одного помещения в другое.

Дозаторы должны быть соответствующим образом поверены (в аккредитованных лабораториях) и промаркированы.

Использованные одноразовые принадлежности (пробирки, наконечники и др.) должны сбрасываться в контейнер для медицинских отходов, содержащий дезинфицирующий раствор (при необходимости).

Поверхности рабочих столов, а также помещения, в которых проводится выделение НК и постановка ПЦР, следует обязательно, до и после проведения работ, облучать с помощью бактерицидных установок в течение 30 минут.

Все поверхности в лаборатории (рабочие столы, штативы, оборудование и др.) ежедневно подвергают влажной уборке с применением дезинфицирующих/моющих средств, регламентированных санитарными правилами и нормами СанПиН 3.3686-21.

ВНИМАНИЕ! Утилизировать отходы с продуктами ПЦР необходимо только в закрытом виде. Не допускается открывать пробирки после амплификации, так как это может привести к контаминации продуктами ПЦР (МУ 1.3.2569-09).

При использовании набора реагентов в клинико-диагностической лаборатории образуются отходы класса В, которые утилизируются в соответствии с требованиями санитарных правил и норм СанПиН 2.1.3684-21 «Санитарно-эпидемиологические требования к содержанию территорий городских и сельских поселений, к водным объектам, питьевой воде и питьевому водоснабжению, атмосферному воздуху, почвам, жилым помещениям, эксплуатации производственных, общественных помещений, организации и проведению санитарно-противоэпидемических (профилактических) мероприятий».

Опасные компоненты в наборе реагентов

Компонент набора реагентов	**Наличие/отсутствие опасного компонента**		**Указание на риски**
	Фасовка S	Фасовка U	
Смесь для амплификации, запечатанная парафином	Нет опасных веществ	-	-
Раствор Taq-полимеразы	Нет опасных веществ	-	-
Минеральное масло	Нет опасных веществ	-	-
Смесь для амплификации	-	Нет опасных веществ	-
Полимераза ТехноТаq МАХ	-	Нет опасных веществ	-
ПЦР-буфер	-	Нет опасных веществ	-
Положительный контрольный образец	**Азид натрия менее 0,1%**	**Азид натрия менее 0,1%**	Не классифицируется как опасный для здоровья человека и окружающей среды

При работе с набором реагентов следует использовать средства индивидуальной защиты для предотвращения контакта с организмом человека. После окончания работы тщательно вымыть руки. Избегать контакта с кожей, глазами и слизистыми оболочками.

При использовании по назначению и соблюдении мер предосторожности контакт с организмом человека исключен.

Не использовать набор реагентов:

- при нарушении условий транспортирования и хранения;
- при несоответствии внешнего вида реагентов, указанного в паспорте к набору реагентов;
- при нарушении внутренней упаковки компонентов набора реагентов;
- по истечению срока годности набора реагентов.

Примечание – Набор реагентов **не содержит** материалов биологического происхождения, веществ в концентрациях, обладающих канцерогенным, мутагенным действием, а также влияющих на репродуктивную функцию человека. При использовании по назначению и соблюдении мер предосторожности является безопасным.

5 ОБОРУДОВАНИЕ И МАТЕРИАЛЫ

При работе с набором реагентов требуются следующие оборудование, реагенты и расходные материалы:

Оборудование, реагенты и расходные материалы	Фасовка S		Фасовка U, дозирование	
	стрипы	пробирки	ручное	автоматизи-рованное
ПЦР-бокс	да	да	да	да
амплификатор с детекцией в режиме реального времени[1]	да	да	да	да[2]
микроцентрифуга-вортекс	да	да	да	да
ротор для микроцентрифуги-вортекса для стрипованных пробирок объёмом 0,2 мл	да[3]	нет	нет	нет
холодильник с морозильной камерой	да	да	да	да
штатив «рабочее место» для пробирок объёмом 0,2 мл	нет	да	да[4]	нет
штатив «рабочее место» для стрипованных пробирок объёмом 0,2 мл	да[3]	нет	нет	нет
штатив «рабочее место» для пробирок объёмом 1,5 мл	да	да	да	да
дозаторы механические или электронные переменного объёма одноканальные, позволяющие отбирать объём жидкости от 2,0 до 20 мкл, от 20 до 200 мкл, от 200 до 1000 мкл	да	да	да	да
одноразовые наконечники с фильтром для полуавтоматических дозаторов, свободные от РНКаз и ДНКаз, объёмом 20 мкл	да	да	да	да
одноразовые наконечники для полуавтоматических дозаторов, свободные от РНКаз и ДНКаз, объёмом 200 мкл, 1000 мкл	да	да	да	да
штатив для дозаторов	да	да	да	да
пробирки микроцентрифужные объёмом 1,5 мл с крышками, свободные от РНКаз и ДНКаз	да	да	да	да
пробирки амплификационные объёмом 0,2 мл с крышками, свободные от РНКаз и ДНКаз или микропланшет ПЦР 96 лунок[5]	нет	нет	да	нет
одноразовые перчатки медицинские, без талька, текстурированные	да	да	да	да
ёмкость для сброса использованных наконечников, пробирок и других расходных материалов	да	да	да	да
транспортная среда (при необходимости), рекомендуется использовать Транспортную среду для биопроб СТОР-Ф, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2020/9640	да	да	да	да
физиологический раствор (0,9% NaCl) стерильный (при необходимости)	да	да	да	да
Устройство дозирующее ДТстрим по ТУ 9443-005-96301278-2012 в варианте исполнения 12М1 или 15М1, ООО «НПО ДНК-Технология», Россия, РУ № РЗН 2015/2982, далее по тексту - ДТстрим	нет	нет	нет	да
одноразовые наконечники с фильтром для дозирующего устройства ДТстрим в комплектации *М1, свободные от РНКаз и ДНКаз, объёмом 200 мкл или рекомендованные для аналогичного используемого дозирующего устройства	нет	нет	нет	да

[1] - далее по тексту – детектирующий амплификатор; требуемые параметры детектирующих амплификаторов указаны ниже

[2] - только детектирующий амплификатор «ДТпрайм» (модификация ДТпрайм» *Х*), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229

[3] - не используется для детектирующего амплификатора Rotor-Gene Q

[4] - только при использовании пробирок

[5] - не используется для детектирующих амплификаторов «ДТлайт» и Rotor-Gene Q

Оборудование, реагенты и расходные материалы	**Фасовка S**		**Фасовка U, дозирование**	
	стрипы	пробирки	ручное	автоматизи-рованное
Устройство для запечатывания планшетов ДТпак, ООО «НПО ДНК-Технология», Россия	нет	нет	да[1]	да
центрифуга с RCF (g) не ниже 100, с адаптером для микропланшетов	нет	нет	да[1]	да
полимерная термоплёнка для запечатывания микропланшетов	нет	нет	да[1]	да
микропланшет ПЦР 384 лунки	нет	нет	нет	да
набор/комплект реагентов для выделения НК из биологического материала[2], рекомендуются: - Комплект реагентов для выделения нуклеиновых кислот ПРОБА-НК/ПРОБА-НК-ПЛЮС по ТУ 9398-035-46482062-2009 в форме комплектации: комплект ПРОБА-НК, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2010/08867; - Комплект реагентов для выделения ДНК по ТУ 9398-037-46482062-2009 в форме комплектации: ПРОБА-ГС, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2010/08696; - Набор реагентов для выделения нуклеиновых кислот ПРОБА-МЧ по ТУ 9398-088-46482062-2016 в форме комплектации ПРОБА-МЧ-РАПИД, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2017/5753; - Комплект реагентов для выделения ДНК ПРОБА-РАПИД по ТУ 9398-015-46482062-2008, ООО «ДНК-Технология ТС», Россия, РУ № ФСР 2008/02939; - Набор реагентов для выделения ДНК человека, бактерий, вирусов и грибов из биологического материала человека и культур микроорганизмов (ПРОБА-ОПТИМА[3]) по ТУ 21.20.23-124-46482062-2021, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2022/17496; - Набор реагентов для выделения ДНК ПРОБА-МЧ МАКС по ТУ 21.20.23-106-46482062-2019, ООО «ДНК-Технология ТС», Россия, РУ № РЗН 2021/14391.				
Дополнительно для предобработки и выделения ДНК из биоптатов/пунктатов с использованием комплектов реагентов ПРОБА-НК и ПРОБА-ГС:				
бокс биологической безопасности II класса				
центрифуга для пробирок объёмом 1,5 мл, с RCF(g) не ниже 12000				
термостат твердотельный, поддерживающий температуру 65 °С				
электрический лабораторный аспиратор с колбой-ловушкой для удаления надосадочных жидкостей				
одноразовые наконечники без фильтра, свободные от РНКаз и ДНКаз, для электрического лабораторного аспиратора				
холодильник с морозильной камерой				
микроцентрифуга-вортекс				
пробирки микроцентрифужные объёмом 1,5 мл с крышками, свободные от РНКаз и ДНКаз				
штатив «рабочее место» для пробирок объёмом 1,5 мл				
дозаторы механические или электронные одноканальные с переменным объёмом, позволяющие отбирать объёмы жидкости от 20 до 200 мкл, от 200 до 1000 мкл				
одноразовые наконечники с фильтром для полуавтоматических дозаторов, свободные от РНКаз и ДНКаз, объёмом 200 мкл, 1000 мкл				
штатив для дозаторов				
одноразовые перчатки медицинские, без талька, текстурированные				
ёмкость для сброса использованных наконечников, пробирок и других расходных материалов				
дезинфицирующий раствор				
физиологический раствор (0,9% NaCl) стерильный (при необходимости)				
транспортная среда (при необходимости)				

[1] - при использовании микропланшетов

[2] - возможность использования набора/комплекта реагентов для выделения ДНК Toxoplasma gondii определяется видом биологического материала (7.1)

[3] - для выделения ДНК из крови только в варианте исполнения ПРОБА-ОПТИМА МАКС

Набор реагентов применяется с детектирующими амплификаторами планшетного и роторного типа с системой детекции флуоресцентного сигнала в режиме реального времени, зарегистрированными в установленном порядке в РФ и соответствующими следующим требованиям:

- обеспечивается работа с объемом реакционной смеси 35 мкл (фасовка S) или 18 мкл (фасовка U);
- обеспечивается работа с флуорофорами: Fam, Hex (Vic);
- подогреваемая крышка с температурой более 100 °С;
- скорость нагрева не менее 2 °С/с;
- скорость охлаждения не менее 1 °С/с;
- точность поддержания и однородность температуры не более ± 0,4 °С.

Для работы с набором реагентов валидированы следующие детектирующие амплификаторы:

- Амплификатор детектирующий «ДТпрайм» по ТУ 9443-004-96301278-2010 (модификация «ДТпрайм *М*»), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229, далее по тексту - «ДТпрайм»;
- Амплификатор детектирующий «ДТпрайм» по ТУ 9443-004-96301278-2010 (модификация «ДТпрайм *Х*»), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10229 (только для набора реагентов в фасовке U для автоматизированного дозирования), далее по тексту - «ДТпрайм» в модификации «ДТпрайм *Х*»;
- Амплификатор детектирующий «ДТлайт» по ТУ 9443-003-96301278-2010 (модификация «ДТлайт *S*»), ООО «НПО ДНК-Технология», Россия, РУ № ФСР 2011/10228 (только для набора реагентов в фасовке S; в фасовке U для ручного дозирования при использовании пробирок), далее по тексту - «ДТлайт»;
- Прибор для проведения полимеразной цепной реакции в режиме реального времени Rotor-Gene Q, QIAGEN GmbH, Германия, РУ № ФСЗ 2010/07595 (только для набора реагентов в фасовке S, пробирки; в фасовке U для ручного дозирования при использовании пробирок), далее по тексту - Rotor-Gene Q;
- Термоциклер для амплификации нуклеиновых кислот 1000 с модулем реакционным оптическим CFX96 (Optical Reaction Module CFX96), Био-Рад Лабораториез, Инк; США, РУ № ФСЗ 2008/03399, далее по тексту - CFX96;
- Амплификатор нуклеиновых кислот Applied Biosystems QuantStudio 5 с гибридизационно-флуоресцентной детекцией продуктов ПЦР в режиме реального времени, «Лайф Текнолоджис Холдингс Пте. Лтд.», Сингапур, РУ № РЗН 2019/8446, далее по тексту - Applied Biosystems QuantStudio 5.

По вопросам применения детектирующих амплификаторов, не указанных выше, требуется согласование с производителем набора реагентов.

6 АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1 Материал для исследования

Для исследования используют кровь, спинномозговую жидкость, амниотическую жидкость, биоптаты или пунктаты из очагов поражения органов и тканей.

6.2 Общие требования

6.2.1 Исследование методом ПЦР относится к прямым методам лабораторного исследования, поэтому взятие биологического материала человека необходимо проводить из места локализации инфекционного процесса. Решение о необходимости исследовать ту или иную локализацию принимает лечащий врач на основании собранного анамнеза и клинической картины заболевания.

6.2.2 Для получения корректных результатов большое значение имеет качество взятия образца биоматериала для исследования, его хранение, транспортирование и предварительная обработка.

При необходимости взятия биоматериала из нескольких биотопов повторите процедуру, каждый раз забирая материал в новую пробирку.

Неправильное взятие биоматериала может привести к получению недостоверных результатов и, вследствие этого, необходимости его повторного взятия.

6.2.3 На этапе подготовки биоматериала используйте одноразовые наконечники с фильтром, свободные от РНКаз и ДНКаз.

6.2.4 Для предотвращения контаминации открывайте крышку только той пробирки, в которую будете вносить биологический материал, и закрывайте ее перед работой со следующей пробиркой.

Примечание – Взятие, предварительную обработку, хранение и перевозку, передачу исследуемого материала в другие организации осуществляют согласно инструктивно-методическим документам, регламентирующим выполнение исследований в соответствии с требованиями МУ 1.3.2569-09 и СанПиН 3.3686-21.

6.3 Взятие материала на исследование

ВНИМАНИЕ! Перед выделением ДНК может потребоваться подготовка образцов биологического материала (6.5).

6.3.1 Периферическая кровь

Взятие материала проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

Ограничение метода[1]: внутривенные инъекции гепарина, инфузии препаратов для парентерального питания – менее чем за 6 часов до исследования.

[1] - если это не противоречит требованиям к используемым наборам/комплектам реагентов для выделения НК

6.3.2 Спинномозговая жидкость, амниотическая жидкость

Взятие материала проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

6.3.3 Биоптаты/пунктаты

Взятие материала проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

При использовании для выделения ДНК комплектов реагентов ПРОБА-НК и ПРОБА-ГС:

Взятие материала проводится в одноразовые пробирки объёмом 1,5 мл с транспортной средой, предназначенной производителем для транспортирования и хранения образцов биологического материала для ПЦР-исследований.

6.4 Транспортирование и хранение образцов биологического материала

Условия транспортирования и хранения образцов биологического материала определяются инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1) или используемых для транспортирования и хранения образцов транспортных сред.

Допускается хранение образцов биологического материала при температуре от 2 °С до 8 °С не более 24 ч. В случае невозможности доставки материала в лабораторию в течение суток допускается однократное замораживание материала. Допускается хранение замороженного материала при температуре от минус 22 °С до минус 18 °С в течение одного месяца (если это не противоречит требованиям к используемым наборам/комплектам реагентов для выделения НК или используемым для транспортирования и хранения образцов транспортным средам).

ВНИМАНИЕ! Следует избегать повторного замораживания и оттаивания образцов.

6.5 Подготовка биологического материала человека для выделения ДНК

6.5.1 Периферическая кровь, спинномозговая жидкость, амниотическая жидкость

Подготовка биологического материала (при необходимости) проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

6.5.2 Биоптаты/пунктаты

Подготовка биологического материала (при необходимости) проводится в соответствии с инструкциями по применению используемых наборов/комплектов реагентов для выделения НК (7.1).

При использовании для выделения ДНК комплектов реагентов ПРОБА-НК и ПРОБА-ГС:

6.5.2.1 Встряхните пробирки с биоматериалом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 3-5 с.

6.5.2.2 Удалите надосадочную жидкость.

Образец готов для выделения ДНК (7.2).

7 ПРОВЕДЕНИЕ АНАЛИЗА

7.1 Выделение ДНК из биологического материала

Для выделения ДНК рекомендуется использовать наборы/комплекты реагентов, имеющие регистрационные удостоверения на медицинское изделие и предназначенные для соответствующих видов биоматериала с целью последующего исследования ДНК методом ПЦР, например, ПРОБА-НК, ПРОБА-ГС, ПРОБА-МЧ-РАПИД, ПРОБА-РАПИД, ПРОБА-ОПТИМА, ПРОБА-МЧ МАКС (таблица 2).

Таблица 2 – Наборы/комплекты реагентов, рекомендованные для выделения ДНК для дальнейшего исследования с использованием набора реагентов Toxoplasma gondii

Набор/комплект реагентов, РУ	Биоматериал	Минимальное количество элюата, мкл
Комплект реагентов ПРОБА-НК, РУ № ФСР 2010/08867	Спинномозговая жидкость, биоптаты, пунктаты	50
Комплект реагентов ПРОБА-ГС, РУ № ФСР 2010/08696	Спинномозговая жидкость, биоптаты, пунктаты	100
Набор реагентов ПРОБА-МЧ-РАПИД, РУ № РЗН 2017/5753	Спинномозговая жидкость, амниотическая жидкость	100
Комплект реагентов ПРОБА-РАПИД, РУ № ФСР 2008/02939;	Спинномозговая жидкость	500
Набор реагентов ПРОБА-ОПТИМА, РУ № РЗН 2022/17496	Цельная периферическая кровь[1]	100
	Спинномозговая жидкость, амниотическая жидкость, биоптаты/пунктаты	400
Набор реагентов ПРОБА-МЧ МАКС, РУ № РЗН 2021/14391	Цельная периферическая кровь	50

Выделение ДНК проводят в соответствии с инструкцией по применению используемого набора/комплекта реагентов.

ВНИМАНИЕ!

1. Одновременно с выделением ДНК из биологического материала необходимо подготовить отрицательный контрольный образец и провести его через все этапы пробоподготовки. Для этого рекомендуется использовать физиологический раствор или отрицательный контрольный образец, входящий в состав набора/комплекта реагентов для выделения нуклеиновых кислот в объёме, указанном в инструкции по применению соответствующего набора/комплекта реагентов.
2. Выделение ДНК из биоптатов/пунктатов с использованием комплектов реагентов ПРОБА-НК и ПРОБА-ГС проводится согласно данной инструкции (7.2).

7.2 Выделение ДНК из биоптатов/пунктатов с использованием комплектов реагентов ПРОБА-НК и ПРОБА-ГС

7.2.1 Общие требования

7.2.2.1 Используйте одноразовые наконечники с фильтром, свободные от РНКаз и ДНКаз. Используемые наконечники меняйте при каждом удалении раствора из

[1] - только с использованием набора реагентов в варианте исполнения ПРОБА-ОПТИМА МАКС

пробирки. При работе с аспиратором используйте наконечники без фильтра, свободные от РНКаз и ДНКаз.

7.2.2.2 При добавлении раствора в пробирку, содержащую биологический материал, вносите раствор аккуратно, не касаясь стенок пробирки. При касании стенки пробирки смените наконечник.

7.2.2.3 Для предотвращения контаминации открывайте крышку только той пробирки, в которую будете вносить раствор или из которой будете удалять надосадочную жидкость, и закрывайте ее перед работой со следующей пробиркой. Не допускается работать одновременно с несколькими пробирками с открытыми крышками.

7.2.2.4 Неизвестные образцы и отрицательный контрольный образец (К-) необходимо обрабатывать по единой схеме одновременно согласно данной инструкции.

7.2.2 Выделение ДНК из биоптатов/пунктатов с использованием комплекта реагентов ПРОБА-НК

ВНИМАНИЕ!

1. Перед началом работы необходимо:

- включить термостат для прогревания до 65 °С;

- достать из холодильника комплект реагентов для выделения нуклеиновых кислот и проконтролировать отсутствие осадка в лизирующем растворе. В случае выпадения осадка необходимо прогреть флакон с лизирующим раствором на термостате, предварительно прогретом до 65 °С, до полного растворения осадка. Затем следует перемешать раствор переворачиванием флакона вверх дном 5-10 раз, избегая пенообразования. Перед использованием охладите раствор до комнатной температуры (от 18 °С до 25 °С). Осадок также можно растворить при комнатной температуре (от 18 °С до 25 °С) в течение приблизительно 12 часов.

2. При прогревании пробирок возможно открывание крышек! Следует использовать пробирки с защёлкивающимися крышками (например, Eppendorf Safe-Lock Tubes) или программируемые термостаты с прижимной крышкой (например, термостат твердотельный программируемый малогабаритный ТТ-1-«ДНК-Техн.», производства ООО «НПО ДНК-Технология»).

7.2.2.1 Промаркируйте по одной одноразовой пластиковой пробирке объёмом 1,5 мл для каждого неизвестного образца и для отрицательного контрольного образца (К-).

7.2.2.2 Добавьте в пробирки с подготовленными образцами биоптатов/пунктатов (см.6.5.2) и в пробирку «К-» по 300 мкл лизирующего раствора, не касаясь края пробирок.

7.2.2.3 Внесите в пробирку, промаркированную «К-», 100 мкл отрицательного контрольного образца. Плотно закройте пробирки, встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.2.4 Термостатируйте пробирки при 65 °С в течение 30 мин, центрифугируйте на микроцентрифуге-вортексе в течение 3-5 с.

7.2.2.5 Перенесите в соответствующие промаркированные пробирки для неизвестных образцов надосадочную жидкость. Для пробирки «К-» переносить надосадочную жидкость не требуется.

7.2.2.6 Добавьте в каждую пробирку по 400 мкл реагента для преципитации, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.2.7 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение 15 мин.

7.2.2.8 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.2.9 Добавьте к осадку по 500 мкл промывочного раствора №1, не касаясь края пробирки, закройте пробирки и аккуратно переверните пробирки 3-5 раз.

7.2.2.10 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение 5 мин.

7.2.2.11 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.2.12 Добавьте к осадку по 300 мкл промывочного раствора №2, не касаясь края пробирки, закройте пробирки и аккуратно переверните пробирки 3-5 раз.

7.2.2.13 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение 5 мин.

7.2.2.14 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.2.15 Откройте пробирки и высушите осадок при 65 °С в течение 5 мин.

7.2.2.16 Добавьте к осадку по 50 мкл буфера для растворения, закройте пробирки.

7.2.2.17 Осадите капли центрифугированием пробирок на микроцентрифуге-вортексе в течение 1-3 с.

7.2.2.18 Термостатируйте пробирки при 65 °С в течение 10 мин. Встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.2.19 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение 30 с.

Препарат ДНК готов к внесению в реакционную смесь для ПЦР.

Препарат ДНК можно хранить при температуре от минус 22 °С до минус 18 °С не более одного месяца или при температуре от минус 72 °С до минус 68 °С не более одного года.

Перед использованием препарата ДНК для постановки ПЦР после хранения необходимо разморозить препарат ДНК и отрицательный контрольный образец при комнатной температуре (от 18 °С до 25 °С) или при температуре от 2 °С до 8 °С, затем встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Для препарата ДНК допускается только однократное размораживание!

Препарат ДНК готов к внесению в реакционную смесь для ПЦР.

7.2.3 Выделение ДНК из биоптатов/пунктатов с использованием комплекта реагентов ПРОБА-ГС

ВНИМАНИЕ! Перед началом работы необходимо включить термостат для прогревания до 50 °С.

7.2.3.1 Промаркируйте по одной одноразовой пластиковой пробирке объёмом 1,5 мл для каждого неизвестного образца и отрицательного контрольного образца (К-).

7.2.3.2 Добавьте в пробирки с подготовленными образцами биоптатов/пунктатов (см.6.5.2) и в пробирку «К-» по 150 мкл лизирующего раствора, не касаясь края пробирок.

7.2.3.3 Внесите в пробирку, промаркированную «К-», 50 мкл стерильного физиологического раствора или транспортной среды. Плотно закройте пробирки, встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.4 Термостатируйте пробирки при 50 °С в течение 30 мин, центрифугируйте пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.5 Перенесите в соответствующие промаркированные пробирки для неизвестных образцов надосадочную жидкость. Для пробирки «К-» переносить надосадочную жидкость не требуется.

7.2.3.6 Тщательно ресуспендируйте сорбент на микроцентрифуге-вортексе. Перевернув пробирку вверх дном, убедитесь, что сорбент не залип на дно пробирки. При необходимости повторите перемешивание сорбента.

7.2.3.7 Добавьте во все пробирки (включая «К-») по 20 мкл предварительно ресуспендированного сорбента, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.8 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение одной минуты.

7.2.3.9 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.3.10 Добавьте к осадку по 200 мкл промывочного раствора №1, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.11 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение одной минуты.

7.2.3.12 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.3.13 Добавьте к осадку по 200 мкл промывочного раствора №2, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.14 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение одной минуты.

7.2.3.15 Добавьте к осадку по 200 мкл промывочного раствора №3, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 3-5 с.

7.2.3.16 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение одной минуты.

7.2.3.17 Не задевая осадок, полностью удалите надосадочную жидкость (отдельным наконечником из каждой пробирки).

7.2.3.18 Откройте крышки пробирок и высушите осадок при 50 °С в течение 5 мин.

7.2.3.19 Добавьте к осадку по 100 мкл элюирующего раствора, не касаясь края пробирки, закройте пробирки и встряхните пробирки на микроцентрифуге-вортексе в течение 5-10 с.

7.2.3.20 Термостатируйте пробирки при 50 °С в течение 5 мин.

7.2.3.21 Центрифугируйте пробирки при RCF(g) 12000 - 16000 в течение одной минуты.

Надосадочная жидкость, содержащая выделенную ДНК, готова к внесению в реакционную смесь для ПЦР.

Полученный препарат ДНК можно хранить до 7 суток при температуре от 2 °С до 8 °С. Перед использованием препарата ДНК для постановки ПЦР необходимо повторить 7.2.3.20 - 7.2.3.21.

Если образец предполагается хранить более 7 суток, перенесите надосадочную жидкость в отдельную одноразовую пробирку.

Более 7 суток препарат ДНК следует хранить при температуре от минус 22 °С до минус 18 °С.

Срок хранения препарата ДНК при температуре от минус 22 °С до минус 18 °С - не более 6 месяцев.

В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, перед использованием препарата ДНК для постановки ПЦР после хранения необходимо разморозить препарат ДНК и отрицательный контрольный образец при комнатной температуре (от 18 °С до 25 °С) или при температуре от 2 °С до 8 °С, затем встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Для препарата ДНК допускается только однократное размораживание!

Препарат ДНК готов к внесению в реакционную смесь для ПЦР.

7.3 Подготовка и проведение ПЦР. Фасовка S

ВНИМАНИЕ!

1. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!
2. При использовании набора реагентов в варианте исполнения «Фасовка S, стрипы» следует строго соблюдать комплектность стрипов и крышек к ним. Не использовать крышки к стрипам из других наборов реагентов!

7.3.1 Промаркируйте по одной пробирке/стрипованной пробирке со смесью для амплификации, запечатанной парафином, для каждого неизвестного образца, для отрицательного контрольного образца (К-) и для положительного контрольного образца (К+).

ВНИМАНИЕ! Количество реагентов рассчитано не более чем на 24 постановки при условии вариабельного количества неизвестных образцов, 1 отрицательного контрольного образца и 1 положительного контрольного образца в каждой постановке.

Пример:

Необходимо проанализировать 4 неизвестных образца. Для этого нужно промаркировать 4 пробирки для неизвестных образцов, одну пробирку для «К-» и одну пробирку для «К+». Общее количество пробирок – 6.

7.3.2 Встряхните пробирку с раствором Taq-полимеразы на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.3.3 Добавьте во все промаркированные пробирки, не повреждая слой парафина, по 10 мкл раствора Taq-полимеразы.

ВНИМАНИЕ! При использовании для проведения ПЦР детектирующего амплификатора Rotor-Gene Q минеральное масло в пробирки не вносится!

7.3.4 Добавьте в каждую пробирку (при необходимости) по одной капле (около 20 мкл) минерального масла. Неплотно прикройте пробирки/стрипы крышками.

7.3.5 Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Перед внесением в пробирки с реакционной смесью для препарата ДНК и отрицательного контрольного образца необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-НК, ПРОБА-РАПИД, ПРОБА-ГС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) встряхните пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с
3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, ПРОБА-МЧ МАКС необходимо, не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки с препаратом ДНК в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.
4. Для предотвращения контаминации следует перед внесением ДНК открывать крышки только тех пробирок, в которые будет вноситься данный образец, и закрывать их, перед внесением следующего. В случае использования стрипов следует закрывать крышку стрипа после внесения в него образцов перед началом работы со следующим. Закрывайте пробирки/стрипы плотно. Препараты ДНК и контрольные образцы следует вносить наконечниками с фильтром.

7.3.6 Внесите в соответствующие промаркированные пробирки, не повреждая слой парафина, по 5,0 мкл выделенного из образцов препарата ДНК. В пробирки, промаркированные «К-» и «К+», ДНК не вносится.

7.3.7 Внесите в пробирку, промаркированную «К-», не повреждая слой парафина, 5,0 мкл отрицательного контрольного образца, прошедшего этап выделения ДНК (см. 7.1).

7.3.8 Внесите в пробирку, промаркированную «К+», не повреждая слой парафина, 5,0 мкл положительного контрольного образца.

7.3.9 Центрифугируйте все пробирки/стрипы на микроцентрифуге-вортексе в течение 3-5 с (при использовании для проведения ПЦР детектирующего амплификатора Rotor-Gene Q центрифугирование не обязательно).

7.3.10 Установите все пробирки/стрипы в детектирующий амплификатор.

7.3.11 Для детектирующих амплификаторов серии ДТ:

Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительных контрольных образцов, отметьте расположение пробирок/стрипов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 3.

7.3.12 Для детектирующих амплификаторов Rotor-Gene Q, CFX96 и Applied Biosystems QuantStudio 5:

Проведите ПЦР с учетом объёма реакционной смеси, равного 35 мкл, по программам амплификации, приведённым в таблицах 4, 5, 6 соответственно.

Таблица 3 – Программа амплификации для детектирующих амплификаторов «ДТпрайм», «ДТлайт» (фасовка S)

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	30	1		Цикл
	94	1	30			
2	94	0	30	5		Цикл
	64	0	15		√	
3	94	0	10	45		Цикл
	64	0	15		√	
4	94	0	5	1		Цикл
5	25[2]	…	…	Хранение		Хранение
√ - режим оптических измерений						

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении А) или предоставляется производителем набора реагентов

[2] - допускается хранение при температуре 10 °С

Таблица 4 – Программа амплификации для детектирующего амплификатора Rotor-Gene Q (фасовка S, пробирки)

№ / Cycling	Температура, °C / Temperature	Время, с / Hold Time, s	Количество циклов / 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	
√ - режим оптических измерений, установить измерение флуоресценции (Acquiring) по каналам детекции Green (Fam) и Yellow (Hex) при 57 °C			

Таблица 5 – Программа амплификации для детектирующих амплификаторов CFX96 (фасовки S, U)

№ блока (Step)	Температура, °C	Время, мин: сек	Количество циклов (повторов)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ - режим оптических измерений (Plate Read), установить измерение флуоресценции по необходимым каналам детекции (Fam, Hex) при 64 °C			

Таблица 6 – Программа амплификации для детектирующих амплификаторов Applied Biosystems QuantStudio 5 (фасовки S, U)

Стадия	№ шага	Температура, °C	Время, мин: сек	Количество циклов (повторов)
Стадия удержания	1	80	01:00	1
	2	94	01:30	1
Стадия ПЦР	1	94	0:20	50
	2	64 √	0:20	
√ - сбор данных для необходимых флуорофоров (Fam, Vic (Hex)) включен				

7.4 Подготовка и проведение ПЦР. Фасовка U, ручное дозирование

ВНИМАНИЕ!

1. Для амплификации следует использовать одноразовые амплификационные пробирки объёмом 0,2 мл или микропланшеты ПЦР 96 лунок[1], герметизируемые термопленкой. Не рекомендуется использовать стрипованные пробирки в связи с опасностью постамплификационной контаминации.

2. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!

[1] - для детектирующих амплификаторов «ДТлайт» и Rotor-Gene Q микропланшеты 96 лунок не используются

7.4.1 Промаркируйте необходимое количество одноразовых амплификационных пробирок объёмом 0,2 мл или микропланшет ПЦР 96 лунок для неизвестных образцов, для отрицательного контрольного образца (К-) и для положительного контрольного образца (К+).

Примечание – Рекомендуется постановка не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

Пример:

Необходимо проанализировать 4 неизвестных образца. Для этого нужно промаркировать 4 пробирки/ зарезервировать 4 лунки микропланшета для неизвестных образцов, одну пробирку/лунку для «К-» и одну пробирку/лунку для «К+». Общее количество пробирок/лунок – 6.

7.4.2 Встряхните пробирку со смесью для амплификации на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.4.3 Внесите во все промаркированные пробирки/необходимые лунки микропланшета (включая «К-» и «К+») по 6,0 мкл смеси для амплификации.

7.4.4 Встряхните пробирки с ПЦР-буфером и полимеразой ТехноТаq МАХ на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Полимеразу ТехноТаq МАХ необходимо доставать из морозильной камеры непосредственно перед использованием.

7.4.5 Приготовьте смесь ПЦР-буфера с полимеразой ТехноТаq МАХ. Для этого смешайте в отдельной одноразовой пробирке:

- 6,0 х (N+1) мкл ПЦР-буфера,
- 0,3 х (N+1) мкл полимеразы ТехноТаq МАХ,

где N – количество промаркированных пробирок/количество необходимых лунок микропланшета с учётом «К-» и «К+».

Пример:

Необходимо проанализировать 4 неизвестных образца, «К–», «К+». Промаркированных пробирок/необходимых лунок микропланшета – 6. Нужно приготовить смесь ПЦР-буфера и полимеразы ТехноТаq МАХ для 7 (6+1) пробирок/лунок, т.е. 42 мкл ПЦР-буфера + 2,1 мкл полимеразы ТехноТаq МАХ.

7.4.6 Встряхните пробирку с приготовленной смесью ПЦР-буфера и полимеразы ТехноТаq МАХ на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Смесь ПЦР-буфера и полимеразы ТехноТаq МАХ необходимо готовить непосредственно перед использованием.

7.4.7 Добавьте во все промаркированные пробирки/необходимые лунки микропланшета со смесью для амплификации по 6,0 мкл смеси ПЦР-буфера и полимеразы ТехноТаq МАХ. Неплотно прикройте пробирки.

ВНИМАНИЕ! После добавления смеси ПЦР-буфера и полимеразы ТехноТаq МАХ в пробирки/лунки со смесью для амплификации необходимо в течение двух часов выполнить 7.4.8 - 7.4.14.

7.4.8 Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Перед внесением в пробирки/лунки с реакционной смесью для препарата ДНК и отрицательного контрольного образца необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-НК, ПРОБА-РАПИД, ПРОБА-ГС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) встряхните пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с
3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, ПРОБА-МЧ МАКС необходимо не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки с препаратом ДНК в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.
4. Для предотвращения контаминации следует перед внесением ДНК открывать крышки только тех пробирок, в которые будет вноситься данный образец, и закрывать их, перед внесением следующего. Закрывайте пробирки плотно. Препараты ДНК и контрольные образцы следует вносить наконечниками с фильтром.

7.4.9 Внесите в соответствующие промаркированные пробирки/необходимые лунки микропланшета по 6,0 мкл выделенного из образцов препарата ДНК. В пробирки/лунки, промаркированные «К-» и «К+», ДНК не вносится.

7.4.10 Внесите в пробирку/лунку, промаркированную «К-», 6,0 мкл отрицательного контрольного образца, прошедшего этап выделения ДНК (см.7.1).

7.4.11 Внесите в пробирку/лунку, промаркированную «К+», 6,0 мкл положительного контрольного образца.

7.4.12 В случае использования микропланшетов ПЦР 96 лунок:

7.4.12.1 Поместите аккуратно, не встряхивая, микропланшет ПЦР в подложку устройства для запечатывания планшетов ДТпак.

7.4.12.2 Проведите запечатывание микропланшета ПЦР полимерной термоплёнкой согласно руководству по эксплуатации прибора ДТпак.

7.4.12.3 Центрифугируйте микропланшет ПЦР при RCF(g) 100 в течение 30 с.

7.4.13 В случае использования пробирок:

Центрифугируйте все пробирки на микроцентрифуге-вортексе в течение 3-5 с (при использовании для проведения ПЦР детектирующего амплификатора Rotor-Gene Q центрифугирование не обязательно).

7.4.14 Установите все пробирки/микропланшет ПЦР в детектирующий амплификатор и проведите ПЦР (7.4.15, 7.4.16).

7.4.15 Для детектирующих амплификаторов серии ДТ:

Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительного контрольных образцов, отметьте расположение образцов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 7.

7.4.16 Для детектирующих амплификаторов CFX96, Applied Biosystems QuantStudio 5 и Rotor-Gene Q:

Проведите ПЦР с учетом объёма реакционной смеси, равного 18 мкл, по программам амплификации, приведённым в таблицах 5, 6, 8 соответственно.

Таблица 7 – Программа амплификации для детектирующих амплификаторов «ДТпрайм», «ДТлайт» (фасовка U)

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	5	15		Цикл
	94	0	5			
2	94	5	00	1		Цикл
3	94	0	30	5		Цикл
	64	0	15		√	
4	94	0	10	45		Цикл
	64	0	15		√	
5	94	0	5	1		Цикл
6	25[2]	…	…	Хранение		Хранение
√ - режим оптических измерений						

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении Б) или предоставляется производителем набора реагентов

[2] - допускается хранение при температуре 10 °С

Таблица 8 – Программа амплификации для детектирующего амплификатора Rotor-Gene Q (фасовка U)

№ / Cycling	Температура, °C / Temperature	Время, с / Hold Time, s	Количество циклов / 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	
√ - режим оптических измерений, установить измерение флуоресценции (Acquiring) по каналам детекции Green (Fam) и Yellow (Hex) при 57 °C			

7.5 Подготовка и проведение ПЦР. Фасовка U, с использованием дозирующего устройства ДТстрим (только для детектирующего амплификатора «ДТпрайм» в модификации «ДТпрайм *X*»)

ВНИМАНИЕ!

1. Для амплификации следует использовать микропланшеты ПЦР 384 лунки, герметизируемые термоплёнкой.
2. При проведении всех последующих действий следует избегать воздействия прямых солнечных лучей на пробирки со смесью для амплификации!

Примечание – Рекомендуется постановка не менее 5 образцов в одном исследовании (3 неизвестных образца, отрицательный и положительный контрольные образцы).

7.5.1 Встряхните пробирку со смесью для амплификации на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.5.2 Встряхните пробирки с ПЦР-буфером и полимеразой ТехноТаq MAX на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ! Полимеразу ТехноТаq MAX необходимо доставать из морозильной камеры непосредственно перед использованием.

7.5.3 Следуя указаниям ПО дозирующего устройства ДТстрим, приготовьте в отдельной одноразовой пробирке смесь ПЦР-буфера с полимеразой ТехноТаq MAX.

7.5.4 Встряхните пробирку с приготовленной смесью ПЦР-буфера и полимеразы ТехноТаq MAX на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

7.5.5 Встряхните пробирку с положительным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с.

ВНИМАНИЕ!

1. Перед проведением дозирования для препарата ДНК и отрицательного контрольного образца необходимо выполнить рекомендации по использованию препарата ДНК, приведённые в инструкции по применению набора/комплекта реагентов для выделения НК.
2. При использовании для выделения ДНК комплектов реагентов ПРОБА-НК, ПРОБА-РАПИД, ПРОБА-ГС (только в случае, если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки) встряхните пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугируйте на микроцентрифуге-вортексе в течение 1-3 с
3. При использовании для выделения ДНК наборов реагентов ПРОБА-МЧ-РАПИД, ПРОБА-МЧ МАКС необходимо, не встряхивая, центрифугировать пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 1-3 с, затем поместить пробирки с препаратом ДНК в магнитный штатив. В случае если после выделения надосадочная жидкость, содержащая выделенную ДНК, была перенесена в новые пробирки, следует встряхнуть пробирки с препаратом ДНК и отрицательным контрольным образцом на микроцентрифуге-вортексе в течение 3-5 с и центрифугировать на микроцентрифуге-вортексе в течение 1-3 с.

7.5.6 Установите пробирки со смесью для амплификации, со смесью ПЦР-буфера и полимеразы ТехноТаq MAX, с препаратами ДНК, отрицательным контрольным образцом и положительным контрольным образцом, а также микропланшет для ПЦР на рабочий стол ДТстрим и проведите дозирование компонентов согласно руководству по эксплуатации.

7.5.7 Поместите аккуратно, не встряхивая, микропланшет ПЦР в подложку устройства для запечатывания планшетов ДТпак после завершения программы на дозирующем устройстве ДТстрим.

7.5.8 Проведите запечатывание микропланшета ПЦР полимерной термоплёнкой согласно руководству по эксплуатации прибора ДТпак.

7.5.9 Центрифугируйте микропланшет ПЦР при RCF(g) 100 в течение 30 с.

7.5.10 Установите микропланшет ПЦР в блок детектирующего амплификатора.

7.5.11 Запустите программное обеспечение детектирующего амплификатора. При первом проведении ПЦР загрузите соответствующий тест[1]. Далее и при последующих постановках создайте соответствующий протокол исследования: укажите количество и идентификаторы образцов, в том числе отрицательного и положительного контрольных образцов, отметьте расположение образцов на матрице термоблока в соответствии с их установкой и проведите ПЦР. При выборе теста должна отображаться программа, приведённая в таблице 7.

[1] - тест для детектирующих амплификаторов серии ДТ создаётся путём ввода параметров (параметры теста указаны в Приложении Б) или предоставляется производителем набора реагентов

8 РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ АМПЛИФИКАЦИИ

Регистрация сигнала флуоресценции проводится детектирующим амплификатором автоматически во время амплификации.

9 УЧЁТ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

9.1 Учёт результатов амплификации осуществляется автоматически с помощью программного обеспечения, поставляемого с детектирующим амплификатором.

9.2 При использовании детектирующих амплификаторов CFX96 следует использовать регрессионный тип анализа (Cq Determination Mode: Regression), во вкладке «Baseline Subtraction» необходимо выбрать «Baseline Subtraction Curve Fit».

9.3 Интерпретация результатов проводится в соответствии с таблицей 9. Результаты постановки валидны, если выполняются условия интерпретации результатов, полученных для контрольных образцов.

Таблица 9 – Интерпретация результатов ПЦР

Канал детекции		**Интерпретация результата**
Fam/Green (искомая ДНК), Cp/Cq/Ct	**Hex/Yellow/Vic** (внутренний контроль), Cp/Cq/Ct	
Неизвестные образцы		
Указан	Не учитывается	**Обнаружена ДНК *Toxoplasma gondii***
Не указан	**Указан**	Не обнаружена ДНК *Toxoplasma gondii*
Не указан	Не указан	Недостоверный результат
Отрицательный контрольный образец		
Не указан	**Указан**	**Отрицательный результат** Результаты постановки валидны
Положительный контрольный образец		
Указан	Не учитывается	**Положительный результат** Результаты постановки валидны

9.4 Недостоверный результат может быть связан с присутствием ингибиторов в препарате ДНК, полученном из биологического материала; неверным выполнением протокола анализа; несоблюдением температурного режима амплификации и др. В этом случае требуется повторное проведение ПЦР с имеющимся препаратом ДНК, либо повторное выделение ДНК и постановка ПЦР для этого образца, либо повторное взятие биологического материала у пациента (выполняется последовательно).

9.5 При получении положительного результата для отрицательного контрольного образца результаты всей постановочной серии считают недостоверными. В этом случае необходимо проведение специальных мероприятий для выявления и устранения возможной контаминации.

9.6 При получении отрицательного результата для положительного контрольного образца результаты всей постановочной серии считают недостоверными. В этом случае требуется повторная постановка амплификации всей партии образцов.

10 ТРАНСПОРТИРОВАНИЕ, ХРАНЕНИЕ И ЭКСПЛУАТАЦИЯ

10.1 Транспортирование

10.1.1 Транспортирование набора реагентов осуществляют в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера, соответствующей условиям хранения компонентов, входящих в состав набора реагентов.

10.1.2 Фасовка S

Допускается транспортирование набора реагентов в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера от 2°С до 25°С не более 5 суток.

10.1.3 Фасовка U

10.1.3.1 Допускается транспортирование набора реагентов, за исключением полимеразы ТехноТаq MAX, в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера от 2 °С до 25 °С не более 5 суток.

10.1.3.2 Допускается транспортирование полимеразы ТехноТаq MAX в термоконтейнерах с хладоэлементами всеми видами крытого транспорта при температуре внутри термоконтейнера до 25°С не более 5 суток.

10.1.4 Наборы реагентов, транспортированные с нарушением температурного режима, применению не подлежат.

10.2 Хранение

10.2.1 Фасовка S

Все компоненты набора реагентов следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов. Смесь для амплификации, запечатанную парафином, следует хранить в защищённом от света месте.

10.2.2 Фасовка U

10.2.2.1 Все компоненты набора реагентов, за исключением полимеразы ТехноТаq MAX, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов. Смесь для амплификации следует хранить в защищённом от света месте.

10.2.2.2 Полимеразу ТехноТаq MAX следует хранить в морозильной камере при температуре от минус 22 °С до минус 18 °С в течение всего срока годности набора реагентов.

10.2.3 Наборы реагентов, хранившиеся с нарушением регламентированного режима, применению не подлежат.

10.3 Указания по эксплуатации

10.3.1 Набор реагентов должен применяться согласно действующей версии утвержденной инструкции по применению.

10.3.2 Для получения достоверных результатов необходимо строгое соблюдение инструкции по применению набора реагентов.

10.3.3 После вскрытия упаковки компоненты набора реагентов следует хранить при следующих условиях:

- все компоненты набора реагентов, за исключением полимеразы ТехноТаq MAX, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в течение всего срока годности набора реагентов;
- смесь для амплификации и смесь для амплификации, запечатанную парафином, следует хранить в холодильнике или холодильной камере при температуре от 2 °С до 8 °С в защищённом от света месте в течение всего срока годности набора реагентов;
- полимеразу ТехноТаq MAX следует хранить в морозильной камере при температуре от минус 22 °С до минус 18 °С в течение всего срока годности набора реагентов.

10.3.4 Наборы реагентов с истекшим сроком годности применению не подлежат.

11 УКАЗАНИЯ ПО УТИЛИЗАЦИИ

11.1 При использовании набора реагентов в клинико-диагностической лаборатории образуются отходы класса В, которые утилизируются в соответствии с требованиями СанПиН 2.1.3684-21 и МУ 1.3.2569-09.

11.2 Наборы реагентов, пришедшие в непригодность, в том числе в связи с истечением срока годности, повреждением упаковки, подлежат утилизации в соответствии с требованиями СанПиН 2.1.3684-21.

12 ГАРАНТИИ ИЗГОТОВИТЕЛЯ

12.1 Предприятие-изготовитель гарантирует соответствие набора реагентов требованиям технических условий при соблюдении условий транспортирования, хранения и эксплуатации, установленных техническими условиями.

12.2 Срок годности набора реагентов – 12 месяцев при соблюдении всех условий транспортирования, хранения и эксплуатации.

13 РЕМОНТ И ТЕХНИЧЕСКОЕ ОБСЛУЖИВАНИЕ

Набор реагентов предназначен для однократного применения и не подлежит техническому обслуживанию и текущему ремонту.

14 СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ ПРИ МАРКИРОВКЕ НАБОРА РЕАГЕНТОВ

	Медицинское изделие для диагностики *in vitro*
	Температурный диапазон
	Содержимого достаточно для проведения <n> тестов
	Использовать до
	Код партии (серии)
	Дата изготовления
	Обратитесь к инструкции по применению или к инструкции по применению в электронном виде
	Номер по каталогу
	Изготовитель
	Не допускать воздействия солнечного света
	Нестерильно

15 ПЕРЕЧЕНЬ ПРИМЕНЯЕМЫХ НАЦИОНАЛЬНЫХ СТАНДАРТОВ

ГОСТ ISO 14971-2021 Изделия медицинские. Применение менеджмента риска к медицинским изделиям

ГОСТ 15.309-98 Система разработки и постановки продукции на производство. Испытания и приемка выпускаемой продукции. Основные положения

ГОСТ Р 2.105-2019 Единая система конструкторской документации. Общие требования к текстовым документам

ГОСТ Р 15.013-2016 Система разработки и постановки продукции на производство. Медицинские изделия

ГОСТ Р 51088-2013 Медицинские изделия для диагностики ин витро. Реагенты, наборы реагентов, тест-системы, контрольные материалы, питательные среды. Требования к изделиям и поддерживающей документации

ГОСТ Р 51352-2013 Медицинские изделия для диагностики ин витро. Методы испытаний

ГОСТ Р ИСО 15190-2023 Лаборатории медицинские. Требования безопасности

ГОСТ Р ИСО 15223-1-2023 Изделия медицинские. Символы, применяемые для передачи информации, предоставляемой изготовителем. Часть 1. Основные требования

ГОСТ Р ИСО 18113-1-2015 Медицинские изделия для диагностики in vitro. Информация, предоставляемая изготовителем (маркировка). Часть 1. Термины, определения и общие требования

ГОСТ Р ИСО 18113-2-2015 Медицинские изделия для диагностики in vitro. Информация, предоставляемая изготовителем (маркировка). Часть 2. Реагенты для диагностики in vitro для профессионального применения

ГОСТ Р ИСО 23640-2015 Изделия медицинские для диагностики in vitro. Оценка стабильности реагентов для диагностики in vitro

ГОСТ Р 53022.3-2008 Требования к качеству клинических лабораторных исследований, Ч.3. Правила оценки клинической информативности лабораторных тестов.

Примечание – Указанные выше стандарты были действующими на момент утверждения инструкции по применению. В дальнейшем, при пользовании документом, целесообразно проверить действие ссылочных нормативных документов на текущий момент. Если ссылочный документ заменён или изменён, то при применении настоящего документа следует пользоваться заменённым (изменённым документом).

16 АДРЕС ДЛЯ ОБРАЩЕНИЯ

Производство наборов реагентов имеет сертифицированную систему менеджмента качества и соответствует требованиям стандарта систем менеджмента качества ISO 9001 в области разработки, производства и продажи IVD реагентов и приборов для молекулярно-генетической диагностики и другого лабораторного применения и ISO 13485 в области разработки, производства и продажи IVD реагентов и приборов для медицинской молекулярно-генетической диагностики.

Производитель: Общество с ограниченной ответственностью «ДНК-Технология ТС» (ООО «ДНК-Технология ТС»), Россия.

Адрес производителя: 117246, Россия, г. Москва, проезд Научный, д. 20, строение 4.

Место производства:

- ООО «ДНК-Технология ТС», 117246, Россия, г. Москва, проезд Научный, д. 20, строение 4.
- ООО «НПО ДНК-Технология», 142281, Россия, Московская область, г. Протвино, ул. Железнодорожная, д. 3.

По вопросам, касающимся качества набора реагентов, следует обращаться в службу клиентской поддержки.

Служба клиентской поддержки:

8(800) 200-75-15 (для России, звонок бесплатный),

+7(495) 640-16-93 (для стран СНГ и зарубежья, звонок платный),

E-mail: hotline@dna-technology.ru

www.dna-technology.ru

Приложение А

Параметры теста, которые необходимо внести в программное обеспечение детектирующих амплификаторов «ДТпрайм», «ДТлайт» при использовании набора реагентов Toxoplasma gondii в фасовке S

1) Количество пробирок в тесте – 1;
2) Объём реакционной смеси – 35 мкл;
3) В окне «Программа амплификации» ввести следующие параметры:

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	30	1		Цикл
	94	1	30			
2	94	0	30	5		Цикл
	64	0	15		√	
3	94	0	10	45		Цикл
	64	0	15		√	
4	94	0	5	1		Цикл
5	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

4) Внести следующие параметры каналов детекции:

Fam	Hex	Rox	Cy 5	Cy 5.5
Toxoplasma gondii	ВК	-	-	-

[1] - допускается хранение при температуре 10 °С

Приложение Б

Параметры теста, которые необходимо внести в программное обеспечение детектирующих амплификаторов «ДТпрайм», «ДТлайт» при использовании набора реагентов Toxoplasma gondii в фасовке U

1) Количество пробирок в тесте – 1;
2) Объём реакционной смеси – 18 мкл;
3) В окне «Программа амплификации» ввести следующие параметры:

№ блока	Температура, °С	мин	с	Число циклов	Режим оптических измерений	Тип блока
1	80	0	5	15		Цикл
	94	0	5			
2	94	5	00	1		Цикл
3	94	0	30	5		Цикл
	64	0	15		√	
4	94	0	10	45		Цикл
	64	0	15		√	
5	94	0	5	1		Цикл
6	25[1]	…	…	Хранение		Хранение
√ - режим оптических измерений						

4) Внести следующие параметры каналов детекции:

Fam	Hex	Rox	Cy 5	Cy 5.5
Toxoplasma gondii	ВК	-	-	-

[1] - допускается хранение при температуре 10 °С

ДНК-Технология
117587, Россия, г. Москва,
вн. тер. г. муниципальный округ Чертаново Северное,
ш. Варшавское, д. 125Ж, к. 5, этаж 1, пом. 12
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+7 (495) 640-16-93 (для стран СНГ и зарубежья, звонок платный)
E-mail: hotline@dna-technology.ru

DNA-TECHNOLOGY

Nucleic Acid Extraction Kits

"Plus" – larger volume of NA solution

"Rapid" – quick method

"Genetics" – human DNA extraction from whole blood

"DBS" – dried blood spots

Suitable for extraction of AVRI pathogens NA, including SARS-CoV-2

Quick NA extraction: from 15-30 minutes

Manual dosing

Automated dosing

Extraction time depends on biomaterial, number of samples, and equipment being used.

Advertising and information material. For detailed information about the Nucleic Acid Extraction Kit, please refer to the instructions for use

	Sample preparation reagent kit product lines	Sample preparation reagent kits	Line features
	PREP-NA	PREP-NA PREP-NA PLUS PREP-NA-ULTRA PREP-NA-FET PREP-NA-S	Lysis and further NA precipitation
Higher purification degree	PREP-GS	PREP-GS PREP-GS PLUS PREP-GS Genetics	Lysis and further NA sorption
Quick extraction (express method)	PREP-RAPID	PREP-RAPID PREP-RAPID Genetics	Thermocoagulation of impurities
	PREP-OPTIMA	PREP-OPTIMA PREP-OPTIMA MAX	Alkaline cell lysis
Automation	PREP-MB		Lysis with further sorption on paramagnetic nanoparticles
	▸ Automated extraction	PREP-MB-NA-S PREP-MB DWP	PREP-MB DWP and PREP-MB-DBS DWP are compatible with KingFisher Flex (Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)
	▸ Manual or automatic dispensing	PREP-MB MAX PREP-MB RAPID PREP-MB-DBS DWP	
Extraction from whole blood		PREP-RADIP Genetics PREP-GS Genetics PREP-OPTIMA MAX PREP-MB MAX	Suitable for genetic assays
Extraction from cell cultures		PREP-CM PREP-MB RAPID PREP-OPTIMA PREP-OPTIMA MAX	Ability to extract from blood cultures – PREP-CM
Extraction from dried blood spots		PREP-CITO DBS PREP-MB-DBS DWP	Suitable for genetic assays

PREP-NA

Extraction of DNA and RNA (human, microbial and viral) by precipitation

Method: lysis and further NA precipitation

PREP-NA

Extraction time: from 30 min*, from 50 min

Obtained NA sample volume: 50 µl

* for nasopharyngeal and oropharyngeal swabs when using shortened extraction method for PCR detection of AVRI pathogens, including SARS-CoV-2. Details of the method are in the annex to the amplification part of the instruction for SARS-CoV-2 kit.

PREP-NA PLUS

Extraction time: from 30 min*, from 50 min

Obtained NA sample volume: 300 µl

* for nasopharyngeal and oropharyngeal swabs when using shortened extraction method for AVRI Complex. Details of the method are in the annex to the amplification part of the instruction for AVRI Complex kit.

PREP-NA-ULTRA

Viral NA extraction from blood plasma with preconcentration

Extraction time: from 50 min

PREP-NA-FET

Fetal DNA extraction from mother's blood

Extraction time: from 2 hours

PREP-NA-S

Extraction of AVRI pathogens NA, including SARS-CoV-2

Extraction time: from 25 min

BIOMATERIAL	PREP-NA	PREP-NA-PLUS	PREP-NA-ULTRA	PREP-NA-FET	PREP-NA-S
Blood plasma					
Urine					
Feces					
Nasopharyngeal/oropharyngeal smears and swabs					
Scrapes from posterior pharynx					
Phlegm					
Saliva					
Urogenital scrapes					
Prostate fluid					
Ejaculate					
Cerebrospinal fluid					
Milk					

PREP-GS

Extraction of DNA (human and microbial) by sorption with extra purification

Method: lysis and further DNA sorption

PREP-GS

Extraction time: from 40 min
Obtained DNA solution volume: 100 µl

PREP-GS PLUS

Extraction time: from 40 min
Obtained DNA solution volume: 300 µl

PREP-GS Genetics

Extraction time: from 40 min

BIOMATERIAL	PREP-GS	PREP-GS PLUS	PREP-GS Genetics
Whole blood			
Blood plasma			
Urine			
Scrapes from posterior pharynx			
Phlegm			
Saliva			
Urogenital scrapes			
Prostate fluid			
Ejaculate			
Cerebrospinal fluid			
Milk			
Native tissues			

PREP-RAPID

Express method of DNA extraction. Sample transportation and extraction in one tube

Method: thermocoagulation of impurities

PREP-RAPID

Microbial DNA extraction
Extraction time: from 15 min

PREP-RAPID Genetics

Human DNA extraction
Extraction time: from 20 min

BIOMATERIAL	PREP-RAPID	PREP-RAPID Genetics
Whole blood		
Urine		
Scrapes from posterior pharynx		
Saliva		
Urogenital scrapes ♀*		
Prostate fluid		
Cerebrospinal fluid		

* We do not recommend to use PREP-RAPID for DNA extraction from men's urogenital scrapes

PREP-MB

Extraction of DNA and RNA using paramagnetic nanoparticles

Method: lysis and release of NA under the action of guanidine thiocyanate with subsequent sorption on paramagnetic nanoparticles and washing from impurities

PREP-MB RAPID

DNA extraction

Extraction time: from 40 min

PREP-MB-NA-S

DNA and RNA extraction

Extraction time: from 40 min

PREP-MB MAX

DNA extraction

Extraction time: from 60 min

Obtained DNA solution volume: 50-300 µl

BIOMATERIAL	PREP-MB RAPID	PREP-MB MAX	PREP-MB-NA-S
Whole blood			
Urine			
Feces			
Nasopharyngeal/oropharyngeal smears and swabs			
Rectal scrapes			
Urogenital scrapes			
Ejaculate			
Cerebrospinal fluid			
Milk			
Amniotic fluid			
Ascitic fluid			
Cell culture			

PREP-MB

Extraction of DNA and RNA using paramagnetic nanoparticles
on KingFisher (Thermo Fisher Scientific) and Auto-Pure (Allsheng) instruments

Method: lysis and release of NA under the action of guanidine thiocyanate with subsequent sorption on paramagnetic nanoparticles and washing from impurities

PREP-MB DWP

Extraction of DNA and RNA
of AVRI pathogens, including SARS-CoV-2

Total time of preparation for NA extraction and NA extraction from 96 samples: from 40 min

Extraction time: from 20 min

Obtained DNA solution volume: 50-300 µl

Compatible instruments: KingFisher Flex
(Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)

PREP-MB-DBS DWP

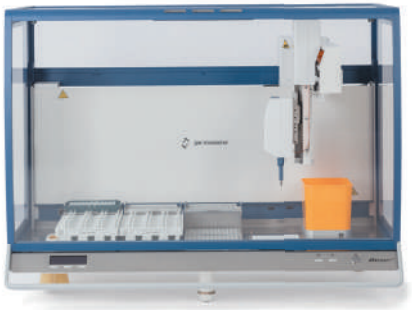
DNA extraction from dried blood spots

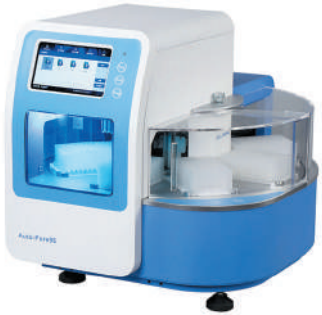
Extraction time: from 60 min

Compatible instruments: KingFisher Flex
(Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)

BIOMATERIAL	PREP-MB DWP	PREP-MB-DBS DWP
Nasopharyngeal, oropharyngeal smears, swabs		
Dried blood spots		

Example of working with a large amount of samples using **PREP-MB DWP** reagent kit





DeepWell preparation:
20 min

DTstream
(DNA-Technology)

RNA extraction:
20 min

KingFisher Flex (Thermo Fisher Scientific)
Auto-Pure 96 (Allsheng)

PREP-OPTIMA

Extraction of DNA (human, microbial and viral).
Universal DNA extraction kit

Method: alkaline cell lysis occurring during thermal incubation

PREP-OPTIMA

Extraction time: from 25 min
Obtained DNA solution volume: 100-450 µl

PREP-OPTIMA MAX

Extraction time: from 25 min
Obtained DNA solution volume: 100-450 µl

BIOMATERIAL	PREP-OPTIMA	PREP-OPTIMA MAX
Whole blood		
Urine		
Feces		
Nasopharyngeal, oropharyngeal smears		
Phlegm		
Rectal scrapes		
Buccal epithelium		
Urogenital scrapes		
Ejaculate		
Milk		
Amniotic fluid		
Synovial fluid		
Native tissues		
Fungal culture		
Bacterial culture		
Cell culture		

PREP-CM

Bacterial and fungal DNA extraction from microbial cultures

Method: alkaline cell lysis occurring during thermal incubation

PREP-CM

Extraction time: from 40 min
Obtained DNA solution volume: 400 µl

BIOMATERIAL	PREP-CM
Fungal culture	
Bacterial culture	
Cell culture	
Blood culture	

PREP-CITO DBS

Human DNA extraction from dried blood spots

Method: alkaline cell lysis occurring during thermal incubation. Removal of possible impurities and stripping of blood from the carrier takes place in the pre-washing stage

PREP-CITO DBS

Extraction time: from 40 min

DNA yield: 30-140 ng when extracted from 10 µl of blood dried on three filter paper discs
Amount of obtained DNA depends on the amount of leukocytes in sample

BIOMATERIAL	PREP-CITO DBS
Dried blood spots	

PREP-L

Lysozyme pretreatment of biomaterial before DNA extraction

Method: enzymatic destruction of peptidoglycans that make up the cell walls of microorganisms, by lysozyme

Pretreatment time:
from 30 min at t= 37 °C
from 60 min at t=18-25 °C

Biomaterial for pretreatment:

- Feces
- Meconium
- Bacterial culture from this biomaterial

Used together with PREP-MB MAX and PREP-NA PLUS NA extraction kits

PREP-FU

Biomaterial pretreatment to obtain lymphocytes from whole blood

Pretreatment time: 1 hour

Biomaterial for pretreatment: whole blood

PREP-PK

Biomaterial pretreatment by proteinase K before nucleic acid extraction

Method: proteolysis by proteinase K and elimination of inhibitory effects

Pretreatment time:

formalin-fixed, paraffin-embedded tissues: DNA — from 150 min,
RNA — from 60 min.

native tissues – 60 min;
cervical scrapes – 90 min.

Biomaterial for pretreatment:

- formalin-fixed, paraffin-embedded tissues (FFPE);
- native tissues;
- cervical scrapes taken into transport-fixating medium for liquid-based cytology

Used together with PREP-NA PLUS nucleic acid extraction kit

PREP-PK reagent kit is not intended for RNA extraction from biomaterial fixated in BD SurePath transport medium.

STOR-F

Transport and storage of human biomaterial

Method: saline solution with the addition of a preservative that prevents the growth of microorganisms

Suitable for further DNA and RNA extraction, including SARS-CoV-2 RNA

Compatible biomaterial:
scrapes/smears of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, eye conjunctiva, skin

Transport and storage of biomaterial:
at t = 2 °C – 8 °C for no longer than 7 days
at t = 18 °C – 25 °C for no longer than 48 hours

STOR-M

Transport and storage of human biomaterial, including those containing mucus

Method: saline solution with mucolytic.
Preservative prevents non-specific microorganisms from reproduction, mucolytic affects disulfide bonds of mucopolysaccharides to thin mucus.

Suitable for further DNA extraction

Compatible biomaterial:

scrapes/smears of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, eye conjunctiva, skin, including those containing mucus

Transport and storage of biomaterial:

at t = 2 °C – 8 °C for no longer than 3 months

at t = 18 °C – 25 °C for no longer than 28 days

BIOMATERIAL	NA extraction reagent kits	DNA		RNA	
		human	microbial	human	microbial
Whole blood	PREP-GS Genetics	■			
	PREP-RAPID Genetics	■			
	PREP-MB MAX	■	■		
	PREP-OPTIMA MAX	■	■		
Blood plasma	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-NA-ULTRA		■		■
	PREP-NA-FET	■			
	PREP-GS		■		
	PREP-GS PLUS		■		
Urine	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-RAPID		■		
	PREP-MB MAX	■	■		
	PREP-GS		■		
	PREP-GS PLUS		■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		
Feces	PREP-NA	■	■	■	■
	PREP-MB MAX	■	■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		
Ejaculate	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-MB MAX	■	■		
	PREP-GS		■		
	PREP-GS PLUS		■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		

BIOMATERIAL	NA extraction reagent kits	DNA		RNA	
		human	microbial	human	microbial
Nasopharyngeal, oropharyngeal smears	PREP-NA				
	PREP-NA-S				
	PREP-MB RAPID				
	PREP-MB-NA-S				
	PREP-MB MAX				
	PREP-GS				
	PREP-GS PLUS				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				
Scrapes from posterior pharynx	PREP-RAPID				
	PREP-NA				
	PREP-NA PLUS				
Saliva	PREP-RAPID				
	PREP-NA				
	PREP-GS				
	PREP-GS PLUS				
Rectal scrapes	PREP-MB RAPID				
	PREP-MB MAX				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				
Urogenital scrapes	PREP-RAPID				
	PREP-NA				
	PREP-NA PLUS				
	PREP-MB MAX				
	PREP-GS				
	PREP-GS PLUS				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				

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DNA-TECHNOLOGY

1143 2025-02-13

CE IVD

For professional use only

PREP-NA DNA/RNA Extraction Kit

PREP-NA PLUS DNA/RNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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Customer service department

REF

P-034-N/1EU
P-036-N/1EU
P-036-N/2EU

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1. INTENDED USE

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** are intended for nucleic acid extraction from human biological material, microbial cultures extracted from this biomaterial, hard ticks (Ixodidae), as well as from biological material of fallen and diseased animals (if necessary) for further PCR/RT-PCR analysis. Human biological material includes: blood (plasma, leukocytes), saliva, phlegm, milk, urine, ejaculate, prostate secretion, liquor, respiratory smears/scrapes, oropharyngeal and nasopharyngeal flushes, smears/scrapes (discharge) from urogenital tract, smears/scrapes from gastrointestinal tract, feces (or meconium), biopsy samples (including formalin-fixed paraffin-embedded (FFPE) samples of tumor tissues), autopsy material, punctate, tissue samples, surgical material (tuberculoma contents), synovial fluid, amniotic fluid, gastric juice, exudates, bronchoalveolar lavage, pleural fluid, endotracheal and nasopharyngeal aspirates, epithelial cell scrapes (discharge) from the conjunctiva of the eye, smears/scrapes from affected skin and erosive-ulcerous elements, nails, vesicular fluid, catheter flushes, endotracheal tube smears and flushes. Biological material from fallen and diseased animals can be used (if necessary): smears and flushes from trachea, nasal cavity, pharynx, cloaca; feces; internal organs.

This medical device is an auxiliary agent in clinical laboratory diagnostics.

The application of the kits does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit.**

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kits** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in biomaterial collection and pretreatment, molecular diagnostics methods and working in the clinical and diagnostic laboratory in the established order: doctor of clinical diagnostic laboratory, medical technologist.

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

2. METHOD

The method is based on lysis and nucleic acid release under the action of a chaotropic agent (guanidine thiocionate), followed by alcohol precipitation and washing from impurities.

3. CONTENT

The detailed description of content is represented in Tables 1-3.

Table 1. The **PREP-NA DNA/RNA Extraction Kit** content for P-034-N/1EU

Reagent	Description	Total volume	Amount
Lysis solution	Light blue foamy liquid	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	45 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	30 mL	1 vial
Dilution buffer	Colorless transparent liquid	5.1 mL (1.7 mL in each)	3 tubes
Negative control	Colorless transparent liquid	3.0 mL (1.5 mL in each)	2 tubes

Table 2. The **PREP-NA PLUS DNA/RNA Extraction Kit** content for P-036-N/1EU, Set No. 1

Reagent	Description	Total volume	Amount
Lysis solution	Light blue foamy liquid	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	45 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	30 mL	1 vial
Dilution buffer	Colorless transparent liquid	30 mL	1 vial
Negative control	Colorless transparent liquid	3.0 mL (1.5 mL in each)	2 tubes

Table 3. The **PREP-NA PLUS DNA/RNA Extraction Kit** content for P-036-N/2EU, Set No. 2

Reagent	Description	Total volume	Amount
Lysis solution	Light blue slightly foaming liquid	15 mL	1 vial
Precipitation buffer	Colorless transparent liquid	20 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	22.5 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	15 mL	1 vial
Dilution buffer	Colorless transparent liquid	15 mL	1 vial
Negative control	Colorless transparent liquid	1.5 mL	1 tube

In the **PREP-NA PLUS DNA/RNA Extraction Kit** the total volume of purified DNA/RNA is larger (300 µL) comparing to standard **PREP-NA DNA/RNA Extraction Kit** (50 µL) for more PCR tests.

The **PREP-NA DNA/RNA Extraction Kit** is designed for NA extraction from 100 test samples (up to 50 runs), including negative controls.

PREP-NA PLUS DNA/RNA Extraction Kit, **set No. 1** is designed for nucleic acid extraction from 100 test samples (up to 50 runs), including negative controls.

PREP-NA PLUS DNA/RNA Extraction Kit, **set No. 2** is designed for nucleic acid extraction from 50 test samples (up to 25 runs), including negative controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs and sterile containers to collect clinical material.

4.2. NA extraction

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator with freezer;
- High speed centrifuge for 1.5 mL tubes (RCF(g) 12,000);
- Solid-state thermostat (temperature at least 65 °C);
- RNase and DNase free 1.5 mL tubes with locking caps (e.g. Eppendorf Safe-Lock tubes);

- Tube rack for 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips for semi-automatic dispensers (volume 20 µL; 200 µL; 1,000 µL);
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution;
- Transport medium for transport and storage of media;
- Physiological saline solution (0.9% NaCl, sterile).

Additional equipment for NA extraction from blood plasma:

- vacuum plastic tubes (Vacuette) with EDTA or sodium citrate;
- centrifuge for Vacuette tubes with RCF(g) at least 900.

Additional equipment for NA extraction from leukocyte blood fraction:

- vacuum 2.0 mL/4.0 mL plastic tubes (Vacuette) with anticoagulant;
- centrifuge for 2.0 mL tubes with RCF(g) at least 50;

Additional equipment for NA extraction from hard ticks (Ixodidae):

- homogenizing rods for 1.5 mL plastic tubes;
- 96% ethanol.

Additional equipment for NA extraction from phlegm (method 1):

- Centrifuge with RCF(g) at least 900;
- 10% trisodium phosphate x 12H2O;
- 1.0M HCl solution;
- 5.0% chloramine solution;
- Distilled water.

Additional equipment for NA extraction from phlegm (method 2):

- Mucolysin.

Additional equipment for NA extraction from FFPE tumor tissues (if necessary):

- **PREP-PK** kit for biomaterial pretreatment during nucleic acid extraction

Additional equipment for NA extraction from feces and bacterial cultures obtained from feces (if necessary):

- **PREP-L** kit for biomaterial lysozyme pretreatment during DNA extraction

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to storage conditions of the kit components.

Kits transported with violation of temperature conditions must not be used.

All components of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** must be stored at temperatures from 2 °C to 25 °C and out of light over the storage period.

A little precipitate is allowed in lysis solution during storage.

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

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

The kit stored under undue regime must not be used.

An expired **PREP-NA DNA/RNA Extraction Kit** must not be used.

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

The conformity of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit.**

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, reverse transcription, 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 the liquid solutions are designed for single use and cannot 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. 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.

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

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 **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** is designed to extract DNA/RNA from peripheral/umbilical blood (plasma and serum, leukocytes), saliva, phlegm, milk, urine, ejaculate, prostate secretion, liquor, respiratory smears/scrapes, oropharyngeal and nasopharyngeal flushes, smears/scrapes (discharge) from urogenital tract, smears/scrapes from gastrointestinal tract, feces (or meconium), biopsy samples (including formalin-fixed paraffin-embedded (FFPE) samples of tumor tissues), autopsy material, punctate, tissue samples, surgical material (tuberculoma contents), synovial fluid, amniotic fluid, gastric juice, exudates, bronchoalveolar lavage, pleural fluid, endotracheal and nasopharyngeal aspirates, epithelial cell scrapes (discharge) from the conjunctiva of the eye, smears/scrapes from affected skin and erosive-ulcerous elements, nails, vesicular fluid, catheter flushes, endotracheal tube smears and flushes. Biological material from fallen and diseased animals can be used (if necessary): smears and flushes from trachea, nasal cavity, pharynx, cloaca; feces; internal organs.

Interfering substances

PCR inhibitors include: hemoglobin and medications present in NA samples due to incomplete removal during NA extraction from the sample, isopropyl alcohol, methyl acetate present in NA samples due to incomplete removal of washout solutions during sample preparation.

Maximum concentrations on interfering substances than can be present in samples without affecting PCR: hemoglobin — 0.35 mg/mL, isopropyl alcohol — 100 µL /mL of NA sample, methyl acetate — 100 µL /mL of NA sample.

When extracting NA from blood plasma, maximum concentrations of interfering substances that did not affect reverse transcription and amplification of laboratory control were: triglycerides — up to 40 mmol/L of plasms, hemoglobin — up to 2.0 g/L, bilirubin — up to 340 µmol/L, crude protein — 60 g/L.

To assess possible interference of medication, the ones that could potentially be residual in human samples from the desired biotopes were selected.

Maximum concentrations on interfering substances than can be present in samples without affecting RT-PCR: whole blood – 5.0% v/v, chlorhexidine (water solution 0.05%), “Lasolvan Rhino” (nasal spray), Rhinofluimucil (nasal spray), Tisin (nasal spray), Oxolin (nasal ointment), Pinosol (nasal drops), Tantum Verde (topical spray), Hexoral (topical aerosol), Berodual (dosed inhalation aerosol), Salbutamol-Teva (dosed inhalation aerosol), Grippferon (dosed nasal spray) – 10% v/v.

General requirements

During biomaterial preparation and MA extraction, use RNase and DNase free single-use tips (filtered, except for supernatant collection using aspirator).

When adding solution into a tube with biomaterial, introduce liquids carefully, without touching the walls of tubes. If touching occurred, change the tip. Tip shall be changed after each removal of solution from the sample.

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

Sample collection

WARNING! Before NA extraction sample pretreatment may be needed.

Sample collection is performed according to Table 4.

In case sample is collected into a transport medium intended for transport and storage of PCR samples, please collect the sample in accordance with instruction to the transport medium.

Transport and storage of samples

Transport and storage of samples is performed according to Table 5.

In case samples are collected into a transport medium intended for transport and storage of PCR samples, transport and storage conditions for the samples are determined by the instruction to the transport medium.

If the study is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transport and storage of the material shall be carried out in accordance with Table 6.

WARNING! Please avoid repeated freezing and thawing of samples!

Table 4. Sample collection for NA extraction

Biomaterial	Method limitations	Features of collection	Order of collection
Amniotic fluid	-	Amniotic fluid is taken during the amniocentesis procedure according to the approved algorithm.	At least 500 µL of amniotic fluid is placed in a dry sterile container with a tightly screw cap. After sample taking close the container tightly.

Biomaterial	Method limitations	Features of collection	Order of collection
Autopsy material Biopsy samples Tissue samples Punctate	-	The material is collected in tubes with transport medium designed by the manufacturer for transport and storage of PCR samples.	Samples with a diameter of no more than 5 mm are placed in tubes with transport medium. After sample intake close the tubes tightly and mark them.
Bacterial cultures	-	Bacterial cultures from liquid and solid media are taken into disposable plastic tubes of 1.5-2.0 mL with 500 µL of sterile physiological saline solution.	Using a disposable microbiological loop or spatula, place a single colony of cells or 100 µL of liquid medium into each tube. After collection, close the tubes tightly and mark them.
Bronchoalveolar lavage Nasopharyngeal and endotracheal aspirates, pleural fluid	-	Bronchoalveolar lavage is taken during the bronchoscopy procedure according to the approved algorithm. The sample is taken into empty disposable tightly screwed tubes with a volume of up to 50 mL.	At least 500 µL of bronchoalveolar lavage is collected in a sterile container. After taking the material, the container is tightly closed. If the RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses is supposed to be detected, each sample is placed in a separate transport container, ensuring the requirements in accordance with the table of guidelines for laboratory diagnosis of COVID-19 (Table 6).
Vesicular fluid Smears/scrapes from affected skin and erosive-ulcerous elements Scrapes of epithelial cells (discharge) from conjunctiva of the eye Exudates	Topical application of medications (sprays, drops, creams and ointments) less than 24 hours before the assay.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution;	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of PCR samples and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe

Biomaterial	Method limitations	Features of collection	Order of collection
		– into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples. **Features of conjunctiva sampling** If there is abundant purulent discharge, it is removed with a sterile cotton swab moistened with physiological saline solution. Discharge/scrape is taken from the inner surface of the lower eyelid by moving to the inner corner of the eye slit. When taking the scrape, hold the eyelid with the hands so that the eyelashes do not touch the probe when blinking.	from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.
Nails	Topical application of medications (creams and ointments) less than 24 hours before the assay.	The material is collected in tubes with transport medium designed by the manufacturer for transport and storage of PCR samples.	Samples is ~2x10 mm in size are placed in tubes with transport medium. After collection, the tubes are tightly closed and marked.
Internal organs of animals	-	Internal organs of animals are taken into a dry sterile container.	Internal organs (trachea and lung fragments, spleen, brain, liver, etc.) are placed in a dry sterile container. After taking the material, the container is tightly closed and marked.
Gastric juice	-	Gastric juice is taken during the gastroscopy according to the approved algorithm.	At least 500 µL of gastric juice is collected in a sterile container. After collection, the container is tightly

Biomaterial	Method limitations	Features of collection	Order of collection
			closed.
Hard ticks (Ixodidae)	-	The tick is collected in a clean container with a tightly closing cap.	Place a piece of damp cloth or water-soaked tissue in the container (to prevent the tick from drying out). Place a live tick in the container.
Liquor	-	Cerebrospinal fluid (liquor) is taken with disposable needles into 1.5 mL tubes according to the established procedure.	At least 500 µL of liquor is collected in a disposable tube. After collection, the tubes are tightly closed and marked.
Endotracheal tube smears and flushes Smears/scrapes of epithelial cells from oropharynx, nasopharynx	Topical application of medications (sprays, drops, creams and ointments) less than 24 hours before the assay. When using aerosols and other forms of medications for inhalation in the treatment of bronchial asthma, samples should be taken no earlier than three hours after inhalation.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples. **Features of nasal smears/scrapes collection** The probe is inserted with a slight movement along the outer wall of the nose to a depth of 2-3 cm to the lower nasal shell. Then the probe is slightly lowered downwards, inserted into the lower nasal passage under the lower	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it. In the case of SARS-CoV-2 and SARS-CoV-like coronaviruses, each biomaterial sample should be placed in a separate transport container, ensuring the requirements listed to Table 6.

Biomaterial	Method limitations	Features of collection	Order of collection
		nasal shell, rotated and removed along the outer nasal wall. **Features of oropharyngeal smears/scrapes collection** Smears/scrapes are taken with a rotating motion from the surface of the tonsils, palatine glands and posterior pharyngeal wall.	
Milk	-	Breast milk is collected in a sterile container with a hermetically closing cap. The period of milk collection is up to 24 hours. Storage during the entire collection period at a temperature of 2 ° C to 8 ° C.	After collection is complete, the milk is mixed and 1.0 mL of material is transferred to a 1.5 mL plastic tube.
Phlegm	-	Material is taken into single-use graduated sterile vials with a wide neck and screw caps with a volume of at least 50 mL.	At least 1.0 mL of phlegm is collected in a vial. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.
Urine	-	Urine is taken into a dry sterile container up to 60 mL in volume with a hermetically screwed cap. **Features of residual urine collection after prostate massage** **WARNING!** If acute prostatitis is suspected, prostate massage is strictly forbidden! Before taking residual	For the assay, the first portion of morning urine is collected in an amount of at least 20 - 30 mL. After urine collection, the container is tightly closed and marked. **Residual urine after prostate massage** 10-15 mL of residual urine is collected by the patient after the

Biomaterial	Method limitations	Features of collection	Order of collection
		urine after prostate massage, sexual abstinence is recommended for three days before the examination. The patient urinates in the toilet, leaving some of the urine in the bladder. Before collecting urine, the head of the penis is treated with a sterile cotton swab moistened with physiological saline solution. The patient is given a massage of the prostate gland for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: if the prostate is soft, gentle pressure is applied, if the prostate is dense, the pressure is increased.	end of the massage. The first portion of urine is collected in a dry sterile container up to 60 mL in volume with a tightly screwed cap. After collection, the container is tightly closed and marked.
Peripheral/umbilical blood	Intravenous injections of heparin, infusion of for parenteral nutrition less than 6 hours before the assay.	Blood is drawn into 2.0 mL/4.0 mL/9.0 mL Vacuette vacuum plastic tubes with EDTA salt added as an anticoagulant at a final concentration of 2.0 mg/mL. Sodium citrate may also be used as an anticoagulant (if it does not contradict the requirements for the PCR reagent kit used together with the reagent kit in all versions). **WARNING!** Heparin as an anticoagulant is not allowed!	To mix the blood with the anticoagulant after sample intake, gently invert the tube at least 3-5 times.
Prostate secretion	-	Before taking the prostate secretion, sexual abstinence is recommended for three days before the assay.	Collection of prostatic secretion is carried out after the end of massage in a 2.0 mL tube or a container up

Biomaterial	Method limitations	Features of collection	Order of collection
		Before collecting the material, the head of the penis is treated with a sterile cotton swab moistened with physiological saline solution. Prostate secretion is collected after preliminary prostate massage through the rectum. The massage is performed by the doctor by means of a vigorous pressure movement from the base to the top of the gland. **WARNING!** If acute prostatitis is suspected, prostate massage is strictly forbidden!	to 60 mL in volume in the form of a free-flowing drop (0.15-1.0 mL). After collection, the container with prostate secretion is tightly closed and marked.
Synovial fluid Saliva	-	Sample is taken in a sterile container.	At least 500 µL of material is collected in a sterile container. After collection, the container is tightly closed and marked.
Oropharyngeal and nasal flushes	-	**Features of oropharyngeal flushes collection** Before taking oropharyngeal flushes, preliminary rinsing of the oral cavity with water is carried out. After that, a thorough rinsing of the oropharynx (for 10-15 s) with 8.0-10 mL of sterile physiological saline solution is carried out. The liquid is collected through a funnel into a sterile tube. It is not allowed to reuse the funnel without prior autoclaving. **Features of nasal flushes collection** The material is taken in a sitting position with the	Transfer 300 µL of the collected fluid into a 1.5 mL plastic tube. If material is taken from several biotopes, it should be transferred to separate tubes. Close the tube tightly and mark it. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.

Biomaterial	Method limitations	Features of collection	Order of collection
		patient's head tilted backwards. To obtain a flush from the nasal cavity, 3.0-5.0 mL of warm sterile physiological saline solution is injected alternately into both nasal passages using a probe or disposable syringe. The flushing fluid from both nasal passages is collected through a funnel into one sterile tube. It is not allowed to reuse the funnel without prior autoclaving. If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material into a new tube.	
Venous catheter flushes	-	Venous catheter flushes are obtained in the laboratory during material treatment.	Cut off 5-10 mm of the catheter tip with sterile scissors and place it in a disposable, empty 1.5 mL plastic tube. Close the tube tightly and mark it.
Endotracheal tubes flushes	-	Sample is taken into a dry sterile 50 mL container with a hermetically screwed cap.	After collecting the material, the tubes are tightly closed and marked. To mix the material, invert the tube 3-5 times. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.
Tuberculoma contents	-	Surgical material is	The contents of the

Biomaterial	Method limitations	Features of collection	Order of collection
		taken into a dry sterile container.	tuberculoma are placed in a dry sterile container. After sampling, the container is tightly closed and marked.
Smears/scrapes from gastrointestinal tract	Use of rectal suppositories, laxatives, medications containing a high percentage of iron, colposcopy — less than 48 hours before the assay	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples.	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.
Smears/scrapes from urogenital tract	Topical application of medications, use of lubricants, vaginal ultrasound — less than 24 hours before the assay.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall

Biomaterial	Method limitations	Features of collection	Order of collection
		manufacturer for transportation and storage of PCR samples. **Features of urogenital scrape collection** Women should not perform genital toilet or sprays the day before the examination. In order to obtain an objective result, it is necessary that the sample contains as many epithelial cells as possible and a minimum amount of mucus and blood. Incorrect sampling may lead to an unreliable result and, therefore, to the need for a second sampling. **WARNING!** Before obtaining a scrape of epithelial cells from the urethra, posterolateral vaginal vault and cervical canal, remove the free-flowing discharge with a sterile cotton swab. **Features of vaginal sampling** The material should be taken before the manual examination. The mirror before manipulation can be moistened with hot water, the use of antiseptics to treat the mirror is contraindicated. The scraping is taken from the posterolateral vaginal vault. In girls, the material is taken from the mucous membrane of the vaginal vestibule, and in some cases from the posterior vaginal	of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.

Biomaterial	Method limitations	Features of collection	Order of collection
		vault through the hymenal rings. **Features of urethral sampling** Before taking the biomaterial, the patient is recommended to refrain from urination for 1.5-2 hours. Immediately before taking the biomaterial it is necessary to treat the external urethral orifice with a tampon, which can be moistened with sterile saline solution. In the presence of purulent discharge it is recommended to take a scrape 15-20 minutes after urination, in the absence of discharge it is necessary to massage the urethra with a probe for biomaterial collection. In women, the probe is inserted into the urethra at a depth of 1.0-1.5 cm; in children, the material for the assay is taken only from the external urethral orifice. **Features of cervical canal sampling** Before sampling remove mucus with a cotton swab and then treat the cervix with sterile physiologic solution. The probe is inserted into the cervical canal to a depth of 0.5-1.5 cm. When removing the probe, avoid touching the vaginal wall. **Features of sampling from the foreskin of the glans of the penis, preputial sac** Before taking the	

Biomaterial	Method limitations	Features of collection	Order of collection
		biomaterial, the patient is recommended to refrain from urination for 1.5-2 hours.	
Animal feces	-	Animal feces are taking into a dry sterile container.	Animal feces in the amount of 4-5 g are placed in a dry sterile container. After material collection, the container is tightly closed and labeled.
Feces (meconium)	-	Fecal or meconium samples with a mass (volume) of approximately 1-3 g (1-3 ml) are used for the assay. The material is collected with a separate filter tip or disposable spatula into a dry sterile vial.	At least 1.0 g of feces (meconium) is placed in a dry sterile vial. After taking the material, the vial is tightly closed and labeled.
Formalin-fixed paraffin-embedded (FFPE) tissues	-	Material collection is performed only by a pathomorphologist. To prepare slices use a clean, sharp microtome blade, cut 2 sections of 10 microns thickness or 3-5 sections of 5 microns thickness from a pre-cut block of paraffin-embedded tissue (approximate cut area 0.5-1.5 cm^2). Paraffin sections are placed in disposable 1.5 mL plastic tubes. The recommended amount of tissue should not be exceeded, as excess paraffin-embedded tissue may reduce the yield of total RNA.	2-4 paraffin slices of 5.0 micron thickness (approximate slice area 0.5-1.5 cm^2) are placed in the tube. After taking the material, the tube is tightly closed and labeled.
Ejaculate	-	Before taking the ejaculate (seminal fluid), sexual abstinence is recommended for three days before the test. Before collecting the ejaculate, the patient urinates in the toilet,	After taking the material, the tube is tightly closed and labeled.

Biomaterial	Method limitations	Features of collection	Order of collection
		emptying the bladder completely. After urination, the patient should wash hands thoroughly with soap and water and toilet the external genitalia with soap and water. The penile head and foreskin should be dried with a sterile tissue. Ejaculate is obtained by masturbation. The ejaculate is collected in a sterile container with a volume of up to 60 mL and a tightly screw cap.	

Table 5. Storage and transport conditions for biological material samples prior to NA extraction

Biomaterial	Transport and storage temperature	Time before NA extraction
Amniotic fluid	from 2 °C to 8 °C	up to 24 hours
Autopsy material	from minus 18 °C to minus 20 °C	up to 7 days
Bacterial cultures	minus 70 °C	prolonged period
Biopsy samples Tissue samples Punctate Oropharyngeal and nasopharyngeal flushes, venous catheter flushes, endotracheal tubes flushes	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6. Note. For bacterial cultures repeated freezing-thawing is allowed.	
Bronchoalveolar lavage	from 2 °C to 8 °C	up to 24 hours
Nasopharyngeal, endotracheal aspirates	from minus 18 °C to minus 20 °C	up to 7 days
Urine Pleural fluid	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	
Vesicular fluid	from 2 °C to 8 °C	up to 24 hours
Smears from endotracheal tubes	from minus 18 °C to minus 22 °C	up to 1 month
Milk Prostate secretion Synovial fluid Saliva Smears/scrapes of epithelial sales from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial cells (discharge) from conjunctiva of the eye Ejaculate	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	

Biomaterial	**Transport and storage temperature**	**Time before NA extraction**
Nails		
Internal organs of animals Gastric juice Tuberculoma contents Animal feces	from 2 °C to 8 °C	up to 24 hours
Hard ticks (Ixodidae)	from 2 °C to 8 °C	up to 48 hours
	WARNING! Transport and storage conditions are intended for a live tick in moistened medium.	
Liquor	from 20 °C to 25 °C	up to 2 hours
	from 2 °C to 8 °C	up to 6 hours
Phlegm Feces (meconium)	from 18 °C to 25 °C	up to 6 hours
	from 2 °C to 8 °C	up to 3 days
	WARNING! If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	
Peripheral/umbilical blood	от 20 °C до 25 °C	up to 2 hours
	from 2 °C to 8 °C	up to 6 hours
	WARNING! Don not freeze whole blood!	
Formalin-fixed paraffin-embedded tissues	from 18 °C to 25 °C	prolonged period
	WARNING! Paraffin melting is not allowed.	

Table 6. Transport and storage conditions for respiratory infections' samples

Sample	Sample collection requirements	Transport	Storage before assay	Notes
Nasopharyngeal and oropharyngeal swab	Plastic tubes and swabs for smears**	4 °C	≤5 days: 4 °C >5 days*: minus 70 °C	Nasopharyngeal and oropharyngeal smears should be placed into one tube to increase viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	A small dilution of the sample is possible
Endotracheal aspirate, nasopharyngeal aspirate or nasal flush	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	-
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	Make sure the material is coming from the lower respiratory tract

* - if it is impossible to store samples at minus 70 °C, store them at minus 20 °C.

** - for transporting samples use transport medium for respiratory smears or physiological solution (if transported to the laboratory no more than 24 hours after specimen collection) or dry probe tampon (if transported to the laboratory no more than 4 hours after sample collection).

Note. STOR-F transport medium is recommended (manufactured by "DNA-Technology TS").

WARNING! Avoid repeated freezing and thawing of samples.

Preparation of samples for NA extraction

Sample preparation is performed in accordance with Table 7.

If the biomaterial samples were taken into the transport medium for transportation and storage of PCR samples, the preparation of the material is carried out in accordance with the instructions for use of the transport medium used for transport and storage of samples.

When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required.

Table 7. Preparation of samples for nucleic acid extraction

Biomaterial	**Sample preparation**
Amniotic fluid Bronchoalveolar lavage Synovial fluid Saliva Liquor Pleural fluid	1. Transfer 500 µL of sample into 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 50 µL in the tube (precipitate + liquid fraction). 4. Add 500 µL of sterile physiological saline solution to precipitate. 5. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 6. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Autopsy material Biopsy samples Tissue samples Punctate	1. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 3-5 seconds. 2. Remove supernatant. Sample is ready for NA extraction.
Bacterial cultures Vesicular fluid Smears from endotracheal tubes Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial	1. Centrifuge the tube with sample in physiological solution/transport medium at RCF(g) 12,000-16,000 for 10 minutes. 2. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.

Biomaterial	Sample preparation
cells (discharge) from conjunctiva of the eye Exudates	
Animal internal organs	1. Place ~250 mg of test sample into a 1.5 mL plastic tube. 2. Add 1.0 mL of sterile physiological saline solution into the tube. 3. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 3-5 seconds. 4. Remove supernatant. Sample is ready for NA extraction.
Gastric juice	1. Transfer 500 µL of gastric juice into a 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Hard ticks (Ixodidae)	1. Place the tick into a 1.5 mL plastic tube. 2. Add 1.0 mL of 96% ethanol into the tube with the tick, shake the tube on vortex for 3-5 seconds, then spin for 3-5 seconds. 3. Remove supernatant as fully as possible. 4. Add 1.0 mL of sterile physiological saline solution, shake the tube on vortex for 3-5 seconds, then spin for 3-5 seconds. 5. Remove supernatant as fully as possible. Sample is ready for NA extraction.
	WARNING! Use pretreated sample for NA extraction immediately.
Phlegm	**Method 1** 1. Transfer 500 µL of phlegm sample into a sterile container. 2. Add to the sample an equal volume of 10% trisodium phosphate x12H2O, close tightly and shake intensely 3. Incubate the mix at 37 °C for 18–24 hours, then neutralize 1 M HCl to pH 6.8-7.4. 4. Spin at RCF(g) 900 for 20 minutes. 5. Drain the supernatant into a container with 5 % chloramine solution for decontamination. 6. Add 500 µL of distilled water, mix by pipetting and transfer to 1.5 mL plastic tube. 7. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 8. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Phlegm	**Method 2** 1. Add mucolysin to the vial with phlegm in proportion 5:1 (5 parts of mucolysin to 1 part of phlegm) based on the vial graduation. 2. Close the vial, shake the mixture and incubate at room temperature (from 18 °C до 25 °C) for 20-30 minutes, shaking the vial every 2-3 minutes. Sample is ready for NA extraction.
Milk	No preparation is required. Sample is ready for NA extraction.
Urine	1. Transfer 1.0 mL of urine into a 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant as fully as possible. 4. Add 1.0 mL of sterile physiological saline solution to the supernatant. 5. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes.

Biomaterial	Sample preparation
	6. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Nasopharyngeal and endotracheal aspirates Flushes from endotracheal tubes	1. Transfer 1.0 mL of biomaterial into a 1.5 mL plastic tube 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Peripheral/umbilical blood	**Obtaining plasma** 1. Spin tubes with blood at RCF(g) 900 at room temperature (from 18 °C to 25 °C) for 20 minutes. 2. After spinning collect upper fraction (plasma) with a pipette and transfer it to a separate 1.5-2.0 mL plastic tube. Sample is ready for NA extraction.
	WARNING! 1. Time between peripheral blood collection and obtaining plasma shall not exceed 6 hours. It is allowed to store plasma at minus 20 °C for up to 3 months (if necessary). 2. Before NA extraction mix the plasm!
	Obtaining leukocyte fraction 1. Transfer 1.5 mL of whole blood into a 2.0 mL plastic tube. 2. Spin the tube with blood at RCF(g) 50 for 10 minutes. 3. After spinning collect 500-600 µL of upper fraction (plasma with leukocytes) with a pipette and transfer to a 1.5-2.0 mL plastic tube. 4. Centrifuge a tube with blood at RCF(g) 10,000 for 10 minutes. 5. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Prostate secretion	1. Prepare the necessary amount of 1.5 mL plastic tubes with 500 µL of sterile physiological saline solution or transport medium for PCR samples. 2. Transfer 100 µL of liquid material into each tube. 3. Centrifuge the tubes at RCF(g) 12,000-16,000 for 10 minutes. 4. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Oropharyngeal and nasal flushes	1. Centrifuge the tube with flush sample at RCF(g) 12,000-16,000 for 10 minutes. 2. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Flushes from	1. Add 100 µL of distilled water or 100 µL of sterile physiological saline

<table>
<tr><th>Biomaterial</th><th colspan="2">Sample preparation</th></tr>
<tr><td>fragments of venous catheter</td><td colspan="2">solution into the tube with fragment of venous catheter.
2. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 1-3 seconds.
Sample is ready for NA extraction.</td></tr>
<tr><td>Tuberculoma contents</td><td colspan="2">Preparation is not required. Sample is ready for NA extraction.</td></tr>
<tr><td rowspan="4">Human feces (meconium), animal feces</td><td colspan="2">Preparation of suspension
1. Prepare the necessary amount of 1.5 mL plastic tubes with 1.0 mL of sterile physiological saline solution
2. Put 0.1-0.2 g (mL) of feces into each tube.
3. Resuspend the tube contents thoroughly on vortex for 5-10 seconds.
4. Centrifuge the tubes with feces suspension at RCF(g) 13,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to precipitate debris to the bottom of the tube.
5. Mark one 1.5 mL tube for each suspension sample.</td></tr>
<tr><td>For bacterial NA extraction:
6. Add the middle fraction from the tubes with feces suspension to the corresponding marked tubes. For this purpose, draw 100 µL of the bacterial fraction (upper white-yellow part of the precipitate formed) from each tube with a separate filter tip. If there is no white-yellow boundary layer between the precipitate and the supernatant, draw 100 µL from the boundary between the precipitate and the supernatant, a partial capture of the precipitate is allowed.
Note. An additional sample preparation using PREP-L reagent kit manufactured by DNA-Technology is allowed.</td><td>For viral NA extraction:
6. Add 100 µL of supernatant from the feces suspension tubes to the corresponding marked tubes.</td></tr>
<tr><td colspan="2">Sample is ready for NA extraction.</td></tr>
<tr><td colspan="2">WARNING! If it is impossible to examine the material within a day and/or if long-term storage is necessary, glycerol at a final concentration of 10-15% is added to the feces suspension in sterile isotonic sodium chloride solution. Samples prepared in this way are frozen only after thorough homogenization and exposure to glycerol for 30-40 minutes.</td></tr>
<tr><td>Formalin-fixed paraffin-embedded tissue</td><td colspan="2">Paraffin-embedded tissue is pretreated using PREP-PK sample pretreatment reagent kit manufactured by DNA-Technology.</td></tr>
<tr><td>Ejaculate</td><td colspan="2">1. Prepare the necessary amount of 1.5 mL plastic tubes with 400-500 µL of sterile physiological saline solution or transport medium for PCR samples.
2. Transfer 100 µL of liquid material into each tube.
3. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin</td></tr>
</table>

Biomaterial	Sample preparation
	for 1-3 seconds. Sample is ready for NA extraction.
Nails taken into STOR-F transport medium	When working with reagent kits for detection of nucleic acids by PCR (manufactured by "DNA-Technology TS", LLC), thermostate the tubes with biomaterial at 95 °C for 10 minutes, shake the tubes on vortex for 3-5 seconds, then spin for 1-3 seconds. 100 µL of biomaterial is used for DNA extraction.

8. PROCEDURE

WARNING!

1. Use RNase- and DNase-free filter tips to introduce and add reagents and samples.
2. When using the aspirator, use RNase- and DNase-free tips without a filter.
3. Change the tips each time the solution is removed from the tube.
4. To prevent contamination, only open and close the cap of the tube you are working with (sample/reagent addition, supernatant removal). Do not handle several tubes with open caps at the same time.
5. When adding solution to a tube containing biological material, introduce the solution carefully without touching the tube walls. If you touch the wall of the tube, change the tip.
   Simultaneously with the NA extraction from biological material it is necessary to prepare a negative control and pass it through all stages of sample preparation.

   For samples of biological material taken in transport medium or physiological solution, or prepared for NA extraction using physiological solution/distilled water, it is recommended to use transport medium or physiological solution/distilled water to prepare a negative control.

   For other samples use negative control (NC) from the reagent kit.

6. Test samples and controls samples must be treated in a single pattern simultaneously according to these instructions.
7. Precipitation is allowed in the lysis solution. In case of precipitation heat the vial with lysis solution on the thermostat previously heated to 65 °C until complete dissolution of the precipitate. Then stir the solution by turning the vial upside down 5-10 times, avoiding foaming. Before use, cool the solutions to room temperature (18 °C to 25 °C). The precipitate can also be dissolved at room temperature (18 °C to 25 °C) for approximately 12 hours.

8. When the tubes are heated, the caps may open! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with a clamp lid (e.g. solid-state programmable small-size thermostat TT-1-"DNA-Technology", manufactured by "DNA-Technology R&D", LLC).

- **Nucleic acid extraction**

Preparation and NA extraction using PREP-NA reagent kit is performed according to Annexes A-D depending on the biomaterial, kit and applied method (Table 8):

Table 8.

Method	Biomaterial
Standard method according to Annex A	Amniotic liquid Bacterial cultures Biological material from fallen and diseased animals (smears and flushes from trachea, nasal cavity, oral cavity, cloaca) Bronchoalveolar lavage Vesicular fluid Gastric juice Liquor Blood leukocytes Smears from endotracheal tubes Phlegm Milk Urine Nasopharyngeal and endotracheal aspirate Blood plasma Pleural fluid Prostate secretion Synovial fluid Saliva Oropharyngeal and nasal flushes, flushes from endotracheal tubes Tuberculoma contents Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial cells (discharge) from conjunctiva of the eye Animal feces Human feces (meconium) Exudates Ejaculate
Short method according to Annex B	Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract Feces (meconium) Ejaculate Nails
Method for biopsy samples according to Annex D	Internal organs of animals Autopsy material Biopsy samples Tissue samples Punctate Flushes from fragments of venous catheter
After pretreatment using PREP-PK **Standard method according to Annex A**	Autopsy material Biopsy samples Tissue samples Punctate FFPE tissue

- **Storage and use of NA preparation**

8.2.1 The NA preparation may be stored at the temperature from 2 °C to 8 °C for no longer than two hours. For long-term storage, the NA preparation should be placed in a freezer and stored at a temperature not exceeding minus 18 °C for no longer than 7 days without thawing before use.

8.2.2 If only PCR DNA testing is intended, the NA preparation may be stored at the temperature from minus 18 °C to minus 22 °C for no longer than one month or at the temperature from minus 68 °C to minus 72 °C for no longer than one year.

WARNING! It is only allowed to thaw NA preparation once.

8.2.3 If the NA preparation has been stored at a temperature not exceeding minus 18 °C, before its use for PCR/RT-PCR it is necessary to thaw the NA preparation and negative control at room temperature (from 18 °C to 25 °C) or at a temperature from 2 °C to 8 °C.

8.2.4 Before using the NA preparation for PCR/RT-PCR after storage and/or thawing, shake tubes with NA preparation and negative control on vortex for 3-5 seconds and spin for 1-3 seconds.

9. SPECIFICATIONS

9.1 Minimum volume of biomaterial for NA extraction:

Biomaterial	Minimum volume
Amniotic liquid, bronchoalveolar lavage, gastric juice, liquor, phlegm (method 1), pleural fluid, saliva, synovial fluid	500 µL
Autopsy material, biopsy samples/punctate, tissue samples	Sample up to 5.0 mm in diameter (50-100 mg)
Prostate secretion, flushes from fragments of venous catheter, tuberculoma contents, ejaculate, milk, blood plasma	100 µL
Bacterial cultures, vesicular fluid, exudates taken into transport medium[1]; Smears from endotracheal tubes taken into transport medium; Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements taken into transport medium; Nails taken into transport medium	100 µL
Internal organs and feces of animals	250 mg
Blood (to obtain leukocyte fraction)	500 µL
Phlegm (method 2), urine, flushes from endotracheal tubes, nasopharyngeal and endotracheal aspirates	1.0 mL
Oropharyngeal and nasal flushes	300 µL
Feces (meconium) for suspension[2]	100 µL/mg
FFPE tissue[3]	2-4 5.0 µm thick paraffin slices (approximate slice area 0.5-1.5 cm^2)

9.2 Functional characteristics of the kit

- purity of nucleic acid samples (A260/280) is 1.4-2.0;
- concentration of nucleic acids in 100 µL of preparation is in the range of 5.9-24.4 ng/µL of NA solution.

9.3 Performance of the kit

- For DNA extraction – 100% (99.05 - 100%) with 95% CI;
- For RNA extraction - 100 % (99.78-100%) with 95% CI.

[1] - DNA-Technology made STOR-F transport medium is recommended

[2] - sample pretreatment using PREP-L is possible

[3] - sample pretreatment using PREP-PK

9.4 Compatible reagent kits:

Nucleic acid extraction kit can be used together with reagent kits for PCR/RT-PCR NA analysis.

10. QUALITY CONTROL

“DNA-Technology Research & Production”, LLC declares that the abovementioned products meet the provision of the Regulation (EU) 2017/746 of the European parliament and of the Council of 5 April 2017. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our official representative in EU by quality issues of **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit**.

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11. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code	VER	Version
NON STERILE	Non-sterile		Caution!
EC REP	Authorized representative in the European Community		Keep away from sunlight

REF P-034-N/1EU
P-036-N/1EU
P-036-N/2EU

VER 1143.2025.02.13

ANNEX A

Standard method of NA extraction from biomaterial[4]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

Note. For samples pretreated with obtaining precipitate and supernatant (see Table 7) mark the tubes with 100 µL of samples prepared for the assay.

2. In case of using this reagent kit together with RT-PCR detection kits ("DNA-Technology TS", "DNA-Technology R&P") that have internal control RNA-IC "A" included, add 10 µL of RNA-IC "A" spun on vortex into the corresponding tubes. Close the tubes.

3. Add 300 µL of lysis solution into each tube. Do not touch the edges of the tube.

4. Add 100 µL of samples to each test tube (except for the tubes with samples with precipitate obtained during pretreatment (see Table 6) and C- tube).

5. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

6. Close the tubes tightly and shake on vortex for 3-5 seconds.

7. Thermostate the tubes at 65 °C for 15 minutes.

8. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

9. Add 400 µL of precipitation buffer into each tube, shake on vortex for 3-5 seconds.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 15 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 5 minutes.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

16. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 5 minutes.

17. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

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

19. Add 50 µL (for PREP-NA) or 300 µL (for PREP-NA PLUS) of dilution buffer to the precipitate.

WARNING! Dilution buffer is <u>different</u> for PREP-NA and PREP-NA PLUS. It is <u>not allowed</u> to use dilution buffer from another version of the kit.

20. Close the tubes and spin down the drops on vortex for 3-5 seconds.

21. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.

22. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to spin down the condensate.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[4] - types of biomaterial are listed in Table 8

ANNEX B

Short method of NA extraction from biomaterial [5]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

Note. For samples pretreated with obtaining precipitate and supernatant (see Table 7) mark the tubes with 100 µL of samples prepared for the assay

2. In case of using this reagent kit together with RT-PCR detection kits ("DNA-Technology TS", "DNA-Technology R&P") that have internal control RNA-IC "A" included, add 10 µL of RNA-IC "A" spun on vortex into the corresponding tubes. Close the tubes.

3. Add 300 µL of lysis solution into each tube. Do not touch the edges of the tube.

4. Add 100 µL of samples to each test tube (except for the tubes with samples with precipitate obtained during pretreatment (see Table 6) and C- tube).

5. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

6. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

7. Thermostate the tubes at 65 °C for 5 minutes.

8. Spin the tubes on vortex for 3-5 seconds.

9. Add 400 µL of precipitation buffer into each tube, shake on vortex for 3-5 seconds.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 10 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

16. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.

17. Remove supernatant, using separate tip for each tube. Do not touch the precipitate. It is allowed to leave up to 20-30 µL of supernatant.

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

19. Add 50 µL (for PREP-NA) or 300 µL (for PREP-NA PLUS) of dilution buffer to the precipitate.

WARNING! Dilution buffer is different for PREP-NA and PREP-NA PLUS. It is not allowed to use dilution buffer from another version of the kit.

20. Close the tubes and spin down the drops on vortex for 3-5 seconds.

21. Thermostate the tubes at 65 °C for 5 minutes. Shake the tubes on vortex for 3-5 seconds.

22. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to spin down the condensate.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[5] - types of biomaterial are listed in Table 8

ANNEX C

NA extraction from hard ticks

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).
2. Add 300 µL of lysis solution into each tube with hard tick (see Table 7) and C-. Do not touch the edges of the tube.
3. Add 100 µL of negative control into the C- tube.
4. Close the tubes tightly and shake on vortex for 3-5 seconds.
5. Thermostate the tubes at 65 °C for 1 hour, spin the tubes on vortex for 3-5 seconds.
6. Rub the tick with a homogenizer rod (separate rod for each tube).
7. Close the tubes tightly, shake on vortex for 3-5 seconds and spin for 3-5 seconds.
8. Transfer supernatant into the corresponding tubes for test samples. Do not transfer the supernatant into the C- tube.
9. Add 400 µL of precipitation buffer into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3-5 seconds.
10. Centrifuge the tubes at RCF(g) 12,000-16,000 for 15 minutes.
11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.
13. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.
14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.
16. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.
17. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
18. Open the tubes and dry the precipitate at 65 °C for 5 minutes.
19. Add 50 µL of dilution buffer to the precipitate, close the tubes.
20. Spin down the drops on vortex for 1-3 seconds.
21. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.
22. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

ANNEX D

NA extraction from biopsy samples and similar types of biomaterials; from venous catheter flushes[6]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

2. Add 300 µL of lysis solution into each tube with samples (see Table 7) and C-. Do not touch the edges of the tube.

3. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

4. Thermostate the tubes at 65 °C according to the table D.1, spin on vortex for 3-5 seconds

Table D.1

Biomaterial	Thermostating time, min
Autopsy material Biopsy samples Internal organs of animals Tissue samples Punctate	30
Flushes from fragments of venous catheter	15

5. Transfer supernatant into the corresponding tubes for test samples. Do not transfer the supernatant into the C- tube.

6. Add 400 µL of precipitation buffer into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3-5 seconds.

7. Centrifuge the tubes at RCF(g) 12,000-16,000 for 15 minutes.

8. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

9. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

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

16. Add 50 µL of dilution buffer to the precipitate, close the tubes.

17. Spin down the drops on vortex for 1-3 seconds.

18. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.

19. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[6] - types of biomaterial are listed in Table 8

600-3 2025-02-13

For professional use only

STOR-M transport medium

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **STOR-M transport medium** is intended for transport and storage of human biological samples (scrapes/swabs of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, skin, conjunctiva of the eye), including those containing an impurity of mucus, followed by nucleic acids analysis (human and microbial DNA, viral RNA) by polymerase chain reaction and reverse transcription (PCR/RT-PCR) methods.

This medical device is an auxiliary agent in clinical laboratory diagnostics.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **STOR-M transport medium.**

The **STOR-M transport medium** can be used in clinical and diagnostic laboratories of medical institutions and 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

The **STOR-M transport medium** is a ready-to-use water-salt transparent, colorless solution with the addition of a preservative, mucolytic and flavoring agent. The preservative prevents the growth of non-specific microorganisms, and mucolytic acts on disulfide bonds of mucopolysaccharides, thereby diluting mucus.

3. CONTENT

The detailed description of content is represented in Tables 1-2.

Table 1. The **STOR-M transport medium** content, package S, 500 for P-910-1/1EU

Reagent	Description	Total volume	Amount
Transport medium	Colorless transparent liquid	50 mL (500 µL in each)	100 tubes

Table 2. The **STOR-M transport medium** content, package S, 1000 for P-911-1/1EU

Reagent	Description	Total volume	Amount
Transport medium	Colorless transparent liquid	100 mL (1.0 mL in each)	100 tubes

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

The kit is intended for single use and designed for 100 tests, including negative controls**.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: sterile single use swabs, cytobrushes, cotton swabs e.t.c for sampling of biomaterial.

4.2. Preparation for NA extraction

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- NA extraction kit;
- High speed centrifuge (RCF(g) no less than 12,000);
- Tube rack for 1.5 mL tubes;
- Container for used pipette tips, tubes and other consumables;
- Single channel pipettes (dispensers covering 100-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 1000 µL);
- Powder-free surgical gloves;
- Disinfectant solution;
- Nucleic acid extraction kit.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **STOR-M transport medium** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to storage conditions of the kit components.

It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C inside the thermoboxes, but for no longer than 14 days.

The kit transported with violation of temperature conditions must not be used.

All components of the **STOR-M transport medium** must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.

The excessive temperature can be detrimental to product performance.

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

The kit stored under undue regime must not be used.

An expired **STOR-M transport medium** must not be used.

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

The conformity of the **STOR-M transport medium** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **STOR-M transport medium.**

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, reverse transcription, 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 the liquid solutions are designed for single use and can not be used more than once. 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 dispensers, 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. 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

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 **STOR-M transport medium** is designed to transport and storage the scrapes/swabs of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, skin, conjunctiva of the eye.

General requirements

Remove free separable mucus with a sterile cotton prior to sampling. In case of sampling from several locations, repeat the procedure several times, each time taking a new swab into new different tube.

To prevent contamination, open the tube, add sample, then close the tube before proceeding to the next sample.

Scrapes/swabs of epithelial cells sampling

Method limitations: local use of medications, vaginal ultrasound less than 24 hours before the test.

WARNING!

1. The use of cytobrushes for urogenital swabs/scrapes is contraindicated in pregnancy.
2. To prevent contamination only open the tube you are working with and close the cap before proceeding to the next tube.

Order of taking:

1. Scrape epithelial cells from the corresponding biotope (i.e. urogenital tract, oropharynx, nasopharynx, rectum, skin, conjunctiva of the eye) with a sterile swab, transfer it to a 1.5 mL plastic tube with STOR-M transport medium and wash it thoroughly for 10-15 seconds. Avoid splashing.
2. Remove swab from solution, press it to the wall of the tube above the solution level and squeeze the rest of the liquid with a rotating liquid. Remove the swab from the tube and dispose of it.
3. Close the tube tightly and mark it.

Transportation and storage of the samples

Transport and store samples in **STOR-M transport medium** for subsequent analysis of human and microbial DNA at temperatures from 2 °C to 8 °C for no longer than 3 months.

Transport and store samples in **STOR-M transport medium** for subsequent analysis of viral RNA at temperatures from 2 °C to 8 °C for no longer than 28 days.

Transport and store samples in **STOR-M transport medium** for subsequent analysis of human and microbial DNA at temperatures from 18 °C to 25 °C is acceptable for no longer than 28 days.

Transport and store samples in **STOR-M transport medium** for subsequent analysis of viral RNA at temperatures from 18 °C to 25 °C is acceptable for no longer than 7 days.

8. PROCEDURE

8.1. General recommendations

1. Use only disposable RNase and DNase free filtered pipette tips.
2. When adding the solution into sample, do not touch the walls of the tubes with the tip. If the tip has touched the wall of the tube, change the tip. Tip should be changed each time when you take out solution from the tube with sample.
3. To prevent contamination, open the tube, add sample/reagent, then close the tube before proceeding to the next sample/reagent.

8.2. Preparation for NA extraction (package S, 500; package S, 1000)

8.2.1 Centrifuge samples in transport medium at RCF(g) 12,000 for 10 minutes.

8.2.2 Remove the supernatant, leaving the approximately 100 µL (precipitate+liquid fraction) in the tube.

WARNING! If for sample pretreatment centrifugation is not needed, p.8.2.1 and 8.2.2 are not performed.

WARNING! For scrape/swab from oropharynx and nasopharynx for subsequent analysis of viral RNA preliminary centrifugation is not required.

8.2.3 Add the lysis buffer in the volume recommended by the NA extraction kit used into the tube with precipitate.

8.2.4 Perform NA extraction according to NA extraction kit user manual.

8.2.5 To make a negative control sample perform p. 8.2.1 and 8.2.2 for tube with **STOR-M transport medium** which does not contain sample, or add 100 µL of STOR-M not containing biomaterial into lysis solution from the NA extraction kit.

The recommended kits for nucleic acids extraction:

- **PREP-RAPID DNA Extraction Kit** ("DNA-Technology Research & Production", LLC, "DNA-Technology TS", LLC, Russia);
- **PREP-GS** and **PREP-GS PLUS DNA Extraction Kits** ("DNA-Technology Research & Production", LLC, "DNA-Technology TS", LLC, Russia);
- **PREP-NA** and **PREP-NA PLUS DNA/RNA Extraction Kits** ("DNA-Technology Research & Production", LLC, "DNA-Technology TS", LLC, Russia);
- **PREP-MB RAPID DNA Extraction Kit** ("DNA-Technology Research & Production", LLC, Russia);
- **PREP-NA-S DNA/RNA Extraction Kit** ("DNA-Technology TS", LLC, Russia).

9. SPECIFICATIONS

The preservation of DNA in biomaterial samples taken into STOR-M (package S, 500) at different storage conditions

Biomaterial	Preservation of DNA (%)	
	28 days under temperatures from 18 °C to 25 °C	3 months under temperatures from 2 °C to 8 °C
scrapes/swabs of epithelial cells from urogenital tract	76.7	67.5
scrapes/swabs of epithelial cells from oropharynx	79.6	68.6
scrapes/swabs of epithelial cells from nasopharynx	64.9	67.6
scrapes/swabs of epithelial cells from conjunctiva of the eye	64.3	51.6
scrapes/swabs of epithelial cells from rectum	86.4	93.1
scrapes/swabs of epithelial cells from skin	83.6	67.1
Total (average, 95% CI)	75.9 (67.8-85.2) n=50	69.2 (57.8-79.4) n=50

The preservation of DNA in biomaterial samples taken into STOR-M (package S, 1000) at different storage conditions

Biomaterial	Preservation of DNA (%)	
	28 days under temperatures from 18 °C to 25 °C	**3 months under temperatures from 2 °C to 8 °C**
scrapes/swabs of epithelial cells from urogenital tract	No losses. The sample is suitable for PCR	No losses. The sample is suitable for PCR
Total (average, 95% CI)	n=25	n=25

The preservation of viral RNA in biomaterial samples taken into STOR-M at different storage conditions

Biomaterial	Preservation of RNA (%)	
	7 days under temperatures from 18 °C to 25 °C	**28 days under temperatures from 2 °C to 8 °C**
scrapes/swabs of epithelial cells from oropharynx (SARS-CoV-2)	No losses. The sample is suitable for RT-PCR	No losses. The sample is suitable for RT-PCR
scrapes/swabs of epithelial cells from nasopharynx (HRV)	No losses. The sample is suitable for RT-PCR	No losses. The sample is suitable for RT-PCR
Total (package S, 500)	n=7	n=9
Total (package S, 1000)	n=7	n=9

10. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the abovementioned products meet the provision of the Regulation (EU) 2017/746 of the European parliament and of the Council of 5 April 2017. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our official representative in EU by quality issues of **STOR-M transport medium.**

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11. KEY TO 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	VER	Version
EC REP	Authorized representative in the European Community	NON STERILE	Non-sterile
	Do not re-use		

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