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INSTRUCTION FOR USE

Reagent Kit for simultaneous qualitative detection of DNA of *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Trichomonas vaginalis* by multiplex real-time polymerase chain reaction "UROGEN-Test-5"

TS 21.20.23-014-97638376-2019

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Contents

Introduction

Urogenital tract infectious diseases cause life quality reduction and decrease in human fertility. Timely diagnosis of urogenital infections helps to reduce the disease burden due to effective drug therapy, which improves the quality of life.

Target analyte. Target analyte detected by UROGEN-Test-5 reagent kit is specific sections of genomic DNA of microorganisms *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium*.

Material for study. DNA samples isolated from patients' biological material – vaginal mucosa smears, endocervical swab, urethral swab, first pass of freely released urine, prostate secretes fluid (if indicated)[1].

Scientific validity of the target analyte is in its specificity (unicity of DNA sequence) in relation to genomes of microorganisms *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium*.

Chlamydia trachomatis are immobile gram-negative bacteria, which are obligate intracellular parasites of eukaryotic cells, belong to the family Chlamydiacea. They have a tropism to the cylindrical epithelium and can affect the mucous membrane of urethra, cervical canal, rectum, oropharynx and eye conjunctiva[2].

Trichomonas vaginalis is a single-celled microorganism, belongs to the protozoan type, family Trichomonadidae, an obligate parasite that receives the most important nutritional components from the secretions of the genital tract by phagocytosis of epithelial cells, and symbiotic microorganisms[3].

Mycoplasma genitalium and *Ureaplasma urealyticum* are conditionally pathogenic microorganisms that can cause urethritis, cervicitis, cystitis, pelvic inflammatory diseases (PID), as well as complications of pregnancy, postpartum and post-abortion complications[4].

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[1] Clinical recommendations: Treatment of patients with sexually transmitted infections and urogenital infections. Russian Society of Dermatovenerologists and Cosmetologists. Moscow. Delovoy Express. - 2012.

[2] Clinical recommendations: Chlamydia infection. Ministry of Health of the Russian Federation, 2016

[3] Clinical recommendations: Urogenital trichomoniasis. Ministry of Health of the Russian Federation, 2016

[4] Clinical recommendations: Urogenital diseases caused by Ureaplasma spp., M. Hominis. Ministry of Health of the Russian Federation. 2016

Neisseria gonorrhoeae is a gram-negative diplococcus, bean-shaped, belongs to the family Neisseriacea. It causes development of infiltrative and degenerative processes of mucous membrane in organs of urogenital and reproductive system, rectum, oropharynx, conjunctiva[5].

Transmission of urogenital infections occurs during sexual contact with an infected person, children can be infected intranatally and through sexual contact, in exceptional cases, young girls can be infected if hygiene rules and childcare rules are violated.

The use area of the reagent kit is clinical laboratory diagnostics, infectious diagnostics.

Indications and Contraindications for Use

Real-time detection of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium* DNA by multiplex PCR is recommended in patients with clinical and/or laboratory signs of inflammation of the organs of the urogenital tract and reproductive system; during pre-conception care; in examination of women during pregnancy (three times: when registering for pregnancy, during 27-30 weeks and during 36-40 weeks of pregnancy); in pregnant women who go into labor without results of an STD examination; in cases with upcoming surgical (invasive) manipulations on genitals and pelvic organs; in persons with a history of perinatal losses and infertility; in sexual partners of patients with STDs; in persons of decreed groups (in accordance with regulatory documents); in persons suffered sexual violence.

The applied DNA detection technique refers to non-invasive procedures, does not pose a threat to human health and does not cause complications.

There are no contraindications for use.

[5] Clinical recommendations: Gonococcal infection. Ministry of Health of the Russian Federation 2016

1. Intended Use

1.1. Intended use: UROGEN-Test-5 reagent kit is designed for qualitative detection of specific sections of genomic DNA of microorganisms *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium* by multiplex allele-specific polymerase chain reaction with hybridization-fluorescence detection in DNA samples to diagnose urogenital infection in patients with clinical and/or laboratory signs of inflammation of the organs of the urogenital tract and reproductive system; during pre-conception care; in examination of women during pregnancy (three times: when registering for pregnancy, during 27-30 weeks and during 36-40 weeks of pregnancy); in pregnant women who go into labor without results of an STD examination; in cases with upcoming surgical (invasive) manipulations on genitals and pelvic organs; in persons with a history of perinatal losses and infertility; in sexual partners of patients with STDs; in persons of decreed groups (in accordance with regulatory documents); in persons suffered sexual violence[1-5].

Biological material for tests:

- vaginal mucosa smears,
- endocervical swab,
- urethral swab,
- first pass of freely released urine,
- prostate secretion.

1.2. Number of Reactions

UROGEN-Test-5 reagent kit is designed in one configuration:

1. **UROGEN-Test-5** to conduct 96 reactions of each multiplex (MU – *Mycoplasma genitalium, Ureaplasma urealyticum;* TN – *Trichomonas vaginalis, Neisseria gonorrhoeae;* Chl – *Chlamydia trachomatis*), which corresponds to testing 90 samples, negative and positive samples with three single runs of 96-well amplifier, or to 32 single tests of samples with negative and positive controls in each testing.

1.3. The use area of the reagent kit is clinical laboratory diagnostics, infectious diagnostics.

1.4. Required qualification: The kit is designed for professional use in medical institutions and clinical diagnostic laboratories. Professional level of potential consumers - doctor of clinical laboratory diagnostics, medical laboratory technician.

1.5. Functional purpose: Test results can be used to diagnose urogenital infections caused by *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium.*

1.6. Indications for Use

Real-time detection of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium* DNA by multiplex PCR is recommended in patients with clinical and/or laboratory signs of inflammation of the organs of the urogenital tract and reproductive system; during pre-conception care; in examination of women during pregnancy (three times: when registering for pregnancy, during 27-30 weeks and during 36-40 weeks of pregnancy); in pregnant women who go into labor without results of an STD examination; in cases with upcoming surgical (invasive) manipulations on genitals and pelvic organs; in persons with a history of perinatal losses and infertility; in sexual partners of patients with STDs; in persons of decreed groups (in accordance with regulatory documents); in persons suffered sexual violence[1-5].

1.7. Demographic and population aspects of the application: without restrictions

1.8. There are no contraindications for use.

1.9. Sterility: the product is not sterile.

2. Method Principle

Method

Real time multiplex allele-specific polymerase chain reaction with hybridization-fluorescence detection.

Type of sample to test

Biological material for tests:

- vaginal mucosa smears,
- endocervical swab,
- urethral swab,
- first pass of freely released urine,
- prostate secretion.

Detection Principle

Real-time qualitative detection of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae,*

Mycoplasma genitalium DNA by multiplex allele specific PCR in DNA sample extracted from biological material includes three stages:

1. PCR preparation;
2. DNA PCR amplification and hybridization-fluorescence detection of amplification products in real-time;
3. Interpretation of results.

DNA samples are used to amplify gene fragments using primers specific to these DNA fragments in the reaction buffer.

PCR buffer contains all the main reagents, including thermally stable hot-start DNA polymerase, dNTP, uracil-DNA glycosidase, and optimized buffer. The presence of uracil-DNA-glycosidase enzyme prevents false positive results from contamination by amplification products, at that the enzyme is completely inactivated during the first cycle of DNA denaturation and does not interfere with amplification of current reaction products.

Primer mix contains fluorescently labeled oligonucleotide probes, which hybridize with complementary fragment of amplified target DNA and destroyed by *Taq* polymerase, as a result, dye and quencher are separated, and fluorescence intensity increases. This allows the accumulation of a specific amplification product to be recorded by measuring the intensity of the fluorescent signal in real time.

The kit contains reagents for multiplex detection of highly specific DNA fragments of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium*, as well as a specific fragment of the human COMT gene (for material collection control, hereinafter-MCC) (Table 1).

Table 1 – Multiplexes included in the kit

Multiplex (primer-mix)	**Channels corresponding to fluorophore**		
	FAM/Green	**ROX/Orange**	**HEX/Yellow**
MU	*Mycoplasma genitalium*	*Ureaplasma urealyticum*	MCC (human *COMT* gene)
TN	*Trichomonas vaginalis*	*Neisseria gonorrhoeae*	MCC (human *COMT* gene)
Chl	*Chlamydia trachomatis*	-	MCC (human *COMT* gene)

MCC allows confirming the fact of material collection from a person, to evaluate quality, effectiveness of DNA isolation and possible

presence of inhibitors in the sample, presence of which can lead to false negative results.

Method Limitation

A possible reason for obtaining a false positive result is contamination at the stage of DNA isolation or multiplex PCR reaction. A false positive result can be detected by using a negative control sample

Breach of the package integrity during transportation.

Use of an expired kit or violation of a kit storage conditions.

Violation of storage conditions when transporting samples.

Multiplex PCR is conducted during 60 minutes (time for sample preparation is not included).

3. Reagent Kit Components

Configuration

UROGEN-Test-5 reagent kit is produced in one configuration: UROGEN-Test-5-96 for 96 reactions of each multiplex (MU – Mycoplasma genitalium, Ureaplasma urealyticum; TN – Trichomonas vaginalis, Neisseria gonorrhoeae; Chl – Chlamydia trachomatis),

Kit Components

Table 2 – Components of UROGEN-Test-5-96 configuration

No.	Reagent	Description	Quantity, Volume
1	PCR-buffer 5x	Transparent colorless liquid	1 test-tube, 1,152 µl
2	Primer-mix MU 2x	Transparent lilac liquid	1 test-tube, 960 µl
3	Primer-mix TN 2x	Transparent lilac liquid	1 test-tube, 960 µl
4	Primer-mix Chl 2x	Transparent lilac liquid	1 test-tube, 960 µl
5	Positive control (PC)	Transparent colorless liquid	1 test-tube, 540 µl
6	Negative control (NC)	Transparent colorless liquid	1 test-tube, 1 500 µl

PCR-buffer5x is ready for use, contains all primary reagents, including a heat-stable hot-start DNA polymerase, deoxynucleotide triphosphates, uracil-DNA glycosidase, and an optimized buffer.

Primer-mix MU 2x is ready to use, contains a multiplex mix of primers and probes:

1. Primers and a probe to a specific fragment of *Mycoplasma genitalium* genomic DNA. Detection is conducted in the FAM/Green channel.

2. Primers and a probe to a specific fragment of *Ureaplasma urealyticum* genomic DNA. Detection is conducted in the ROX/Orange channel.

3. Primers and a probe to MCC. Detection is performed in the HEX/Yellow channel.

Primer-mix TN 2x is ready to use, contains a multiplex mix of primers and probes:

1. Primers and a probe to a specific fragment of *Trichomonas vaginalis* genomic DNA. Detection is conducted in the FAM/Green channel.
2. Primers and a probe to a specific fragment of *Neisseria gonorrhoeae*. genomic DNA. Detection is conducted in the ROX/Orange channel.
3. Primers and a probe to MCC. Detection is performed in the HEX/Yellow channel.

Primer-mix Chl 2x is ready to use, contains a multiplex mix of primers and probes:

1. Primers and a probe to a specific fragment of *Chlamydia trachomatis* genomic DNA. Detection is conducted in the FAM/Green channel.
2. Primers and a probe to MCC. Detection is performed in the HEX/Yellow channel.

The reaction in the HEX/Yellow Ct≤32 indicates a sufficient quality of material collection, efficiency of nucleic acid extraction, and absence of PCR inhibitors. In case there is no reaction in the HEX/Yellow channel or if Ct is greater 32 and if at the same time there is no reaction in the FAM/Green and ROX/Orange specific channels, the result should be considered invalid, and for such test sample, a second study should be conducted, starting with DNA extraction. If the result is invalid again, biomaterial should be collected from this patient for the second time.

Positive control (PC) is ready for use and is a mixture of plasmid DNA with synthetic inserts of amplified DNA fragments: the human COMT gene fragment, specific fragments of *Chlamydia trachomatis*,

Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium DNAs.

Negative control (NC) is ready for use and is deionized water, free of DNase.

The kit contains no medicinal products for medical use, substances of human or animal origin

4. Reagent Kit Characteristics

4.1 Technical and Performance Characteristics

Table 3 – UROGEN-Test-5 reagent kit

Parameter Name	**Characteristics and Standards**
1. Technical Characteristics	
1.1. Appearance	
1.1.1 Configuration UROGEN-Test-5 for 96 reactions of each multiplex (MU – *Mycoplasma genitalium, Ureaplasma urealyticum;* TN – *Trichomonas vaginalis, Neisseria gonorrhoeae;* Chl – *Chlamydia trachomatis*)	
PCR-buffer 5x	Transparent colorless liquid
Primer-mix MU 2x	Transparent lilac liquid
Primer-mix TN 2x	Transparent lilac liquid
Primer-mix Chl 2x	Transparent lilac liquid
PC	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to point 1.4 by TS 21.20.23-014-97638376-2019
1.3. Marking	According to point 4 by TS 21.20.23-014-97638376-2019
1.4. Packaging	According to point 5 by TS 21.20.23-014-97638376-2019
2. Performance Characteristics	
2.1 Positive result with PC	Registration of the fluorescence signal increase in test tubes with PC on the FAM/Green channel Ct≤35, ROX/Orange channel Ct≤35 and HEX/Yellow channel Ct≤35.
2.2 Negative result with NC	In the test tubes with NC on the FAM/Green, HEX/Yellow and ROX/Orange Ct is not specified or >35.

2.3 Passage of the reaction in tubes with a control sample of sensitivity	In tubes with a control sample of sensitivity on the FAM, HEX and ROX Ct 28-35
2.4 Passage of the reaction in tubes with a control sample of specificity.	In tubes with a control sample of specificity on the FAM and ROX channels is not indicated, on the HEX channel Ct 25-30.
3. Analytical characteristics	
Analytical specificity	Specific to DNA of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and to the human COMT gene DNA fragment. Absence of non-specific positive results of amplification in the presence of DNA of *Gardnerella vaginalis*; *Lactobacillus* spp.; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus pyogenes*; *Streptococcus agalactiae*; *Candida albicans*; *Mycoplasma hominis*; *Ureaplasma parvum*; *Neisseria flava*; *Neisseria subflava*; *Neisseria sicca*; *Neisseria mucosa*; *Treponema pallidum*; *Toxoplasma gondii*; *HSV-1 and HSV-2; CMV*; *HPV* in the sample and as well as human DNA in a concentration of up to 10^8 copies/ml of the sample.
Analytical sensitivity	Minimum 500 copies for 1 ml[6] of DNA of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium.*

[6] When for DNA extraction NA-Extra reagent kit (produced by TestGene, LLC) is used, the sample volume is 100 μl, eluate is max. 50μl.

4.2 Clinical Effectiveness

Table 4 – Clinical effectiveness characteristics

Type of testing sample	**Number of tests**	**Diagnostic sensitivity** with 95 % confidence probability	**Diagnostic specificity** with 95 % confidence probability
Vaginal mucosal smears	26	100% (95% diagnostic interval: 96%-100%)	100% (95% diagnostic interval: 96%-100%)
Scraping from the cervical canal	27	100% (95% diagnostic interval: 96%-100%)	100% (95% diagnostic interval: 96%-100%)
Scraping from the urethra	18	100% (95% diagnostic interval: 96%-100%)	100% (95% diagnostic interval: 96%-100%)
Cellular urine sediment	17	100% (95% diagnostic interval: 96%-100%)	100% (95% diagnostic interval: 96%-100%)
Prostate gland fluid	15	100% (95% diagnostic interval: 96%-100%)	100% (95% diagnostic interval: 96%-100%)

5. Risks Associated With the Use of UROGEN-Test-5

The border risk zone includes the following:

1. loss of functional properties of reagents included in the kit due to transportation, storage or operation under inappropriate conditions;
2. contamination of reaction mixtures with test DNA samples by PC test-tube content or PCR products;
3. a test is conducted with the use of a DNA sample of insufficient quality (low concentration and/or poor purification);
4. failure to meet requirements for sample preparation, testing and disposal due to the fact that unqualified personnel work with the kit;
5. use of an unsuitable kit (use after the expiration date or if the packaging is broken).

In the area of the unacceptable zone, no risks were identified.

Total residual risk of using a medical device UROGEN-Test-5 reagent kit for simultaneous qualitative detection of DNA of *Chlamydia trachomatis, Mycoplasma genitalium, Neisseria gonorrhoeae, Ureaplasma urealyticum, Trichomonas vaginalis* by

multiplex real-time polymerase chain reaction is acceptable; the benefit of its use exceeds the risk.

6. Precautions When Working With the Kit

Potential risk Class – 2b – in accordance with Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation dated June 6, 2012 No.4n.

All components and reagents included in UROGEN-Test-5 reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards system. Noxious substances. Classification and general safety requirements".

The reagents included in UROGEN-Test-5 reagent kit have a low vapor elasticity and exclude the possibility of inhalation poisoning.

The reagents included in UROGEN-Test-5 reagent kit are non-toxic, as they are prepared by mixing individual non-toxic components.

The material should be considered as infected or suspected of being infected and handled in accordance with the requirements of sanitary and epidemiological rules for the safety when working with microorganisms in pathogenicity (hazard) groups 3 and 4 (Health Regulations 1.3.3118-13), Methodology Guidelines "Organization of Laboratory Work Using Methods of Nucleic Acid Amplification When Working With Material Containing Microorganisms of Pathogenicity Groups I-IV" (Methodology Guidelines 1.3.2569-09).

Personnel should ensure and comply with the rules of biological safety and requirements for organization of work and conduct it in order to prevent contamination of premises and equipment with nucleic acids and (or) amplicons of tested samples.

The work should be carried out in a laboratory that performs molecular biological (PCR) studies of clinical material in compliance with the Sanitary and Epidemiological Rules and Regulations SanPiN 2.1.7.2790-10 "Sanitary and Epidemiological Requirements for Medical Waste Handling". Personnel should follow recommendations set out in Methodology Guidelines 287-113, Methodology Guidelines 1.3.2569-09.

When working it is required:

- to remove unused reagents in accordance with paragraph 4.28 of SanPiN 2.1.7.2790-10 "Sanitary and Epidemiological Requirements for Medical Waste Handling";

ATTENTION! When removing waste after amplification (test-tubes containing PCR products), it is not allowed to open tubes and spill the content, since this can lead to contamination of a laboratory area, equipment and reagents with PCR products.

1. use the kit strictly for its intended purpose, according to this instruction;
2. only specially trained personnel is allowed to work with the kit (a specialist with higher medical education who has been trained in licensed qualification courses to work with Pathogenic Biological Agents (PBA) of pathogenicity groups III and IV and to conduct PCR diagnostics, a laboratory assistant with secondary special medical education);
3. do not use the kit after the expiration date;
4. avoid contact with skin, eyes and mucous membrane. In case of contact, immediately flush the affected area with water and seek medical assistance.

The precautions are not provided for the effects of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure drops, overloads, or sources of thermal ignition.

The kit contains no substances of human or animal origin that have a potential infectious nature, so precautions against any special, unusual risks when using or selling the product are not provided.

7. Equipment and Materials Required When Working With the Kit

The work with UROGEN-Test-5 reagent kit for multiplex PCR is carried out in working area 3 (for preparing reactions) (Methodology Guidelines 1.3.2569-09).

Multiplex PCR Equipment:

1. PCR-cabinet, biological safety cabinet Class II and III (for example, «BMB-II – «Laminar-S"-1,2», «Laminar Systems", Russia).
2. Vortex (for example, «TETA-2», «Biokom», Russia).

3. Set of electronic or automatic variable volume dispensers (e.g., "Eppendorf", Germany).

4. Refrigerator for +2°C to +8 °C with a freezer for max -16 °C.

5. Amplifier[7] with real-time fluorescent detection via channels corresponding to fluorophore FAM/Green, HEX/Yellow and ROX/Orange, for example, CFX96 (BioRad, USA), DTprime, DTlite (DNA-Technology, Russia), Rotor-Gene Q (Qiagen, Germany), Rotor-Gene 3000 or 6000 (Corbett Research, Australia), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the product:

ATTENTION! When working with DNA, it is required to use only disposable sterile plastic consumables that have a special marking "DNase-free".

1. Disposal pipette tips with an aerosol barrier up to 1,000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA).;
2. Thin-walled disposable PCR test tubes, optically transparent lid:
   - PCR test-tubes, 0.2 ml
   - strip PCR test-tubes, 0.2 ml
   - PCR racks with optically transparent film (e.g., Axygen, USA).
4. Isolation gown coat and disposable talc-free gloves.
5. Container with disinfectant.
6. "Workplace" racks for 0.2 ml test tubes or for 0.2 ml tube strips (e.g., InterLabService, Russia).

8. Test Samples

Test sample type

Biological material for testing:

1. Epithelial cell scrapings (from urethra, cervical canal, posterior vaginal voult);
2. first pass of freely released urine;
3. prostate secretes fluid.

8.1 Biological Sample Collection

[7] Amplifiers must be maintained, calibrated, and used in accordance with the manufacturer's recommendations. The use of this kit while a device out of calibration may have an impact on the performance of the test.

Attention! Before starting work, it is required to study Guidelines "Sampling, Transportation and Storage of Clinical Material for PCR-Diagnostics", developed by the Federal State Budgetary Institution of Science Central Research Institute of Epidemiology of Federal service for surveillance on consumers' rights protection and human well-being (Rospotrebnadzor), Moscow, 2012.

Biological material is collected and packed by healthcare providers specially trained to follow biological safety requirements and rules when working and collecting material suspected of being infected with microorganisms of pathogenicity group III.

Collection of Material for Testing

Scraping of the mucous membranes of the urogenital tract (cervical canal, urethra, and vagina).

The material is collected using special sterile disposable instruments - urogenital probes, cervical cytological brushes or swabs, depending on the source of the clinical material. After the material is collected, the specimen on the end of the probe is placed into 1.5 – 2.0 ml test-tube containing 300 – 500 µl of sterile saline solution, transport medium for transporting and storage of biomaterial for PCR assays.

In case the instruction for use of the reagent kit instructs to place the end of the probe with collected material into a test-tube, place the end of the probe with collected material into the test-tube, leave the end of the probe in the transport medium by snapping off the probe in a specially marked place. In case there is no mark (line for breaking) on the probe or there is no instruction to leave the part of the probe in the test-tube, the probe with collected specimen is placed in the medium and pressed to the inside wall of the test-tube, the probe is rotated for 5-10 seconds, then the probe is removed from the medium and the test-tube is tightly closed. Before nucleic acid extraction procedure, drops of the collected material should be collected from the walls of the tube and inside part of the test-tube lid by centrifuging at 1,500 – 3,000g, carefully vortex the content of the tube, avoiding spillage of the material around and on the inside part of the lid.

Collecting material from urethra

On the day before material collection, female patients should avoid washing their genitals or taking a vaginal douche. To obtain an objective result, it is necessary that the test material contain the largest possible

number of epithelial cells and minimum amount of mucus and blood impurities. Incorrect biomaterial collection can lead to an unreliable result and, as a result, to recollection of biomaterial.

Patients should not have urinated for at least 1.5-2 hours prior to specimen collection. Immediately before biomaterial collection treat the external opening of the urethra with a swab, which is wet with a sterile saline solution.

In the presence of purulent discharge, it is recommended to collect specimens 15-20 minutes after urination, if there is no discharge, it is necessary to massage the urethra with a probe for collecting biomaterial. In case with female patients, the probe is inserted to a depth of 1.0-1.5 cm. In children, the material for the study is collected only from the outer opening of the urethra.

Vaginal swab specimen collection

The material should be taken before manual examination. The speculum can be washed with hot water before manipulation, the use of antiseptics for speculum treatment is not allowed. The vaginal swab is collected from the posterolateral arch of the vagina. In girls, the material is taken from the mucous membrane of the vestibule of the vagina, and in some cases - from the posterior arch of the vagina through the hymenal rings.

Cervical swab specimen collection

Before material collecting, it is necessary to remove the mucus with a cotton swab and then treat the cervix with a sterile saline solution. The probe is inserted into the cervical canal to a depth of 0.5-1.5 cm. When removing the probe, it is necessary to exclude its contact with the walls of the vagina

The limitation of the method is the local use of drugs, ultrasound with a vaginal sensor less than 24 hours before the study.

Urine

For analysis, the first catch of morning urine is collected in an amount of at least 20-30 ml in a special dry sterile 50 ml collection cup.

Pre-treatment of samples

Shake the container with urine. Using a tip with a filter transfer 1 ml of urine into sterile 1.5 ml test tubes, centrifuge for 5 minutes at 10,000 g, in the presence of a large amount of salts, resuspend only top layer of the salt sediment (1 ml) and then concentrate again. Using a vacuum aspirator with a trap flask, completely remove the supernatant, leave the sediment.

Add the transport medium to the sediment to obtain the final 0.2 ml volume, thoroughly mix the contents using vortex.

Prostate secretion

Before prostate secretion collection use sterile cotton wet with saline solution to clean a patient's penis. Prostate secret should be collected after prostate massage performed by pressing on a prostate gland through rectum. The doctor presses the prostate gland several times from the base to the top. Then the prostate secretion is expressed out of the cavernous body, which is collected in a sterile container (vessels with a wide neck, test tubes).

Conditions for transportation, storage and disposal of initial clinical material:

- at +2 … +8 °C – no longer than 24 hours
- at -18 … -22 °C – no longer than a month.

ATTENTION! Avoid repeated freezing and thawing of samples.

Recording, storage, transfer and transportation of biological material with suspected urogenital infections should be carried out in accordance with the requirements of sanitary and epidemiological rules for the safety when working with microorganisms in pathogenicity (hazard) groups 3 and 4 (Health Regulations 1.3.3118-13) and current sanitary rules on the procedure for recording, storage, transfer and transportation of microorganisms of pathogenicity groups I-IV.

Disposal of clinical material (Class B) as extremely epidemiologically hazardous waste is carried out in accordance with SanPiN 2.1.7.2790-10.

8.2 DNA extraction from biological material

The following extraction kits are recommended:

- NA-Extra reagent kit for nucleic acids extraction, produced by TestGene, LLC, Russia,
- PREP-RAPID DNA Extraction Kit according to TS 9398-015-46482062-2008, produced by DNA Technology, LLC, Russia, (Registration certificate No. FSR 2008/02939 of 15.06.2015)
- Reagent kit for nucleic acids extraction (PREP NA/ PREP-NA PLUS) according to TS 9398-035-46482062-2009, produced by DNA Technology LLC, Russia (Registration certificate No. FSR 2010/08867 of 13.10.2016)

- RealBest Extraction 100 reagent kit for nucleic acids extraction produced by AO Vector-Best, Russia (Registration certificate No. RZN 2014/1423 of 25.03.2017)

- RealBest DNA-Express reagent kit for nucleic acids extraction, produced by AO Vector-Best, Russia (Registration certificate No. RZN 2015/2300),

or other similar kits designed for nucleic acids extraction from biological material.

During DNA extraction it is required to strictly follow the protocol and instruction for use for the applied reagent kit.

Conditions for possible storage of tested DNA samples

- at +2 … +8 °C – no longer than 24 hours
- at -18 … -22 °C – no longer than a month
- ai - 80°C – for a long time.

8.3 Interfering substances and restrictions on the use of the testing material

The effect of potentially interfering substances on the performance of UROGEN-Test-5 reagent kit was tested for potentially interfering substances that will occur during collection of biological material samples in the following concentrations:

- Haemoglobin 10%,
- Mucin 5%.

Based on the results of the study, potentially interfering substances encountered during DNA isolation from clinical material, evaluated at concentrations that are expected to occur with normal use of UROGEN-Test-5 reagent kit do not have an interfering effect.

Restrictions on the use of the tested material:

- testing material is not subject to use in case of violation of storage and transportation conditions (temperature, duration, repeated freezing and thawing);

- use of samples contaminated with foreign biological material is not allowed.

9. Testing

It is not required to install, assemble, adjust, or calibrate a medical device before operation.

ATTENTION! When working with DNA, it is required to use only disposable sterile plastic consumables that have a special marking "DNase-free". It is required to use an individual pipette tip with an aerosol barrier for each component.

ATTENTION! The components of the reaction mixture should be mixed according to Table 5 immediately before performing the analysis.

9.1. Preparation of kit components for PCR

Before preparing the reactions, PCR cabinet, equipment and materials contained in it should be wet cleaned and exposed to UV-radiation for 20-30 minutes.

1. Thoroughly mix the contents of the test tubes with the DNA extracted for analysis, PCR buffer, primer mixes, NC and PC, turning each tube up and down 10 times or mixing using vortex at low speed during 3-5 seconds, then remove the drops from the test tube lids by short centrifugation.

2. Prepare the required number of 0.1 – 0.2 test tubes for PCR for each multiplex: number of tested samples[8] + 1 PC + 1 NC.

Depending on the need to identify specific microorganisms and the type of kit configuration used, each sample is placed with one or more multiplexes (primer mixes). Table 8 shows the layout of PCR tubes when using three multiplexes.

[8] To improve accuracy, it is recommended to analyze each sample in two repetitions.

Table 5 – Layout of PCR tubes

Multiplex	Sample 1	Sample n	PC	NC
MU (*Mycoplasma genitalium, Ureaplasma urealyticum*)	○	○	○	○
TN *(Trichomonas vaginalis, Neisseria gonorrhoeae)*	○	○	○	○
Chl *(Chlamydia trachomatis)*	○	○	○	○

For each reaction it is required to use:

1. **PCR-buffer – 4 µl,**
2. **Primer mix (MU, TN or Chl) – 10 µl,**
3. **Sample (tested DNA sample[9], PC, NC) – 6 µl.**

Total reaction amount – 20 µl.

ATTENTION! It is not allowed to change the reaction amount. When the amount is changed, the sensitivity of the method decreases dramatically!

9.2 PCR Protocol

Reaction test-tubes should be prepared in accordance with Table 5 in the following order:

1. Label PCR 0.2 ml test tubes. For each multiplex take the required number of test-tubes for testing samples + 1 PC + 1 NC (Table 6).

2. Add 4µl of PCR buffer to each test-tube[9].

3. Add 10 µl of each primer-mix (MU, TN, or Chl) in the test tubes for each multiplex (Table 9)[8].

4. Add 6 µl of the extracted DNA to the appropriate test tubes for the test samples [10]. Do not add DNA to PC and NC test tubes.

[9] It is recommended to first prepare a mixture of primer mix and PCR buffer for each multiplex in a separate 1.5-2.0 ml tube at the rate of: (n+3)x4 µl PCR buffer + (n+3) x10 µl of the corresponding primer mix, where n is the number of samples. Mix using vortex, remove drops by short centrifugation and add 14 µl to each PCR tubes for the corresponding multiplex according to Table 9.

[10] To prevent PCR inhibition, the sample amount can be reduced to 1-5 µl, while the reaction volume is increased to 20 µl by deionized water from NC.

6. Add 6 µl of PC to the test tubes with used multiplexes.

7. Add 6 µl of NC to the test tubes with used multiplexes.

8. Centrifuge the test tubes during 1-3 seconds to remove the drops from the walls. Use a microcentrifuge-vortex.

9. Place the test tubes in the reaction module of the real-time PCR device. It is recommended to place the test tubes in the center of the thermal block to evenly press the test tubes with the heating cap.

10. Program the device to perform the appropriate amplification and detection of fluorescent signal, follow the user manual supplied with the device. PCR protocol is shown in Table 6.

11. Specify the number and identifiers of the samples; mark the layout of the test tubes on the thermal block matrix in accordance with their layout.

Table 6 – PCR Protocol

Stage	Temperature, ˚C	Time, min:sec	Detection channels	Total cycles
1	95	02:00	-	1
2	95	00:15	-	5
	64	00:15		
3	95	00:05	-	40
	64	00:15	FAM/Green, ROX/Orange, HEX/Yellow	

12. Be sure that the FAM/Green, HEX/Yellow, and ROX/Orange detection channels are used in the optical measurement parameters of the amplification program.

13. Start PCR with fluorescent signal detection.

14. At the end of the program, start performing the data analysis.

10. Registration and Interpretation of Results

The results are recorded automatically after the PCR is completed using the software of the device.

Recommendations for setting the cycle threshold

For all models of amplifiers, the cycle threshold is set individually for each channel at a level corresponding to 10-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

The results are interpreted using Ct values of the FAM/Green, ROX/Orange, and HEX/Yellow channels (Table 7).

First, the reaction rate and Ct values in control samples are evaluated. The results in tested samples are tested only if the correct results with PC and NC are obtained.

Table 7 – Results interpretation in the FAM/Green, ROX/Orange and HEX/Yellow

Multiplex (primer-mix)	**Channel corresponding to the fluorophore**		
	FAM/Green	**ROX/Orange**	**HEX/Yellow**
MU	*Mycoplasma genitalium*	*Ureaplasma urealyticum*	MCC (human *COMT* gene)
TN	*Trichomonas vaginalis*	*Neisseria gonorrhoeae*	MCC (human *COMT* gene)
Chl	*Chlamydia trachomatis*	-	MCC (human *COMT* gene)

Interpretation of results in control samples

For negative and positive controls, the following results should be obtained (Table 8).

Table 8 – Test results for PC and NC

Material added	Selected fluorophore		
	FAM/Green (*Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma genitalium*)	HEX/ Yellow (KBM)	ROX/Orange (*Ureaplasma urealyticum, Neisseria gonorrhoeae*)
NC	Ct >35 or 0	Ct >35 or 0	Ct >35 or 0
PC	Ct ≤35	Ct ≤35	Ct ≤35

When negative control values differ from those indicated in Table 12, the results of the entire assay are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

When positive control values differ from those indicated in Table 11, repeated amplification of the entire batch of samples are required. If after repeated amplification positive control values differ from those indicated in Table 11, the reagents must be replaced.

Interpretation of results in testing samples

Methods of result interpretation are shown in Tables 9-11.

Table 9 – Method of result interpretation for multiplex MU (*Mycoplasma genitalium, Ureaplasma urealyticum)*

Ct Values		Result
Specific channels **(FAM/Green, ROX/Orange)**	MCC channel KBM **(HEX/Yellow)**	
Ct FAM/Green ≤35	not counted	DNA of *Mycoplasma genitalium is* **detected**
Ct FAM/Green absent	Ct ≤32	DNA of *Mycoplasma genitalium is* **not detected**
Ct FAM/Green >35	not counted	The result for DNA of *Mycoplasma genitalium* presence **is doubtful**
Ct ROX/Orange ≤35	not counted	DNA of *Ureaplasma urealyticum is* **detected**
Ct ROX/Orange - absent	Ct ≤32	DNA of *Ureaplasma urealyticum is* **not detected**
Ct ROX/Orange >35	not counted	The result for DNA of *Ureaplasma urealyticum* is **doubtful**
Ct in both specific channels >35 or 0	Ct>32 or 0	**Invalid** result

Table 10 – Method of result interpretation for multiplex TN (*Trichomonas vaginalis, Neisseria gonorrhoeae)*

Ct Values		Result
Specific channels **(FAM/Green, ROX/Orange)**	MCC channel **(HEX/Yellow)**	
Ct FAM/Green ≤35	not counted	DNA of *Trichomonas vaginalis* ***is*** **detected**
Ct FAM/Green - absent	Ct ≤32	DNA of *Trichomonas vaginalis* **is not detected**
Ct FAM/Green >35	not counted	The result for DNA of *Trichomonas vaginalis* presence **is doubtful**
Ct ROX/Orange ≤35	not counted	DNA of *Neisseria gonorrhoeae* ***is*** **detected**

Ct ROX/Orange - absent	Ct ≤32	DNA of *Neisseria gonorrhoeae* ***is*** **not detected**
Ct ROX/Orange >35	not counted	The result for DNA of *Neisseria gonorrhoeae* **presence is doubtful**
Ct in both specific channels >35 or 0	Ct>32 or 0	**Invalid** result

Table 11 – Method of result interpretation for multiplex Chl (*Chlamydia trachomatis)*

Ct Values		Result
Specific channel **(FAM/Green)**	MCC channel **(HEX/Yellow)**	
Ct FAM/Green ≤35	not counted	DNA of *Chlamydia trachomatis* ***is*** **detected**
Ct FAM/Green - absent	Ct ≤32	DNA *Chlamydia trachomatis* ***is*** **not detected**
Ct FAM/Green >35	not counted	The result for *Chlamydia trachomatis* presence **is doubtful**
Ct >35 or 0	Ct>32 or 0	**Invalid** result

If an increase in the fluorescence of a specific product through the FAM/Green or ROX/Orange channels is registered for a biological sample before cycles 24 (Ct<24), this indicates a high initial DNA concentration of the corresponding pathogen. In this case, it is possible to obtain a false negative result for the pathogen, whose DNA is present in a low concentration, through the second channel. To exclude false-negative results, it is recommended to repeat the PCR for the isolated DNA sample.

Invalid results can be caused by a low concentration of DNA, presence of inhibitors in the DNA sample extracted from clinical material, incorrect performance of the assay protocol, non-compliance with the temperature regime of PCR, etc.

In the case of an invalid and doubtful result, the conclusion is not issued, it is necessary to re-collect the biomaterial from the patient and re-test it.

If a questionable result is repeated, repeat the study with a reagent kit from another manufacturer or by another method.

11. Medical Device Stability Information

Period of validity of the kit is 12 months.

Period of validity of the kit after opening is 12 months.

12. Storage, Transportation and Usage Conditions

Storage

UROGEN-Test-5 in the manufacturer's package should be stored at - 20 °C during the entire period of validity of the kit; it is allowed to store the kit at +2 …+8 °C up to 90 days.

It is allowed to freeze / thaw UROGEN-Test-5 kit maximum 10 times.

After opening, store in the same conditions as before opening.

A reagent kit stored in violation of storage conditions cannot be used.

Transporting

UROGEN-Test-5 reagent kit can be transported by all types of covered vehicles in accordance with the transport rules applicable to this type of transport.

It is allowed to transport UROGEN-Test-5 reagent kit at a temperature -20 °C during the entire period of validity of the kit. It is allowed to transport UROGEN-Test-5 reagent kit at a temperature +2 …+8 °C during max 30 days or at ambient temperatures but not higher +30 °C and no longer than 5 days.

Atmospheric pressure is not controlled, because it does not affect the quality of the product.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared refrigerating elements. The type, volume and number of icepacks put in the cold box of thermal container with reagent kits, and the thermal container size varies according to the duration and conditions of transportation.

Reagent kits transported with violation of temperature conditions cannot be used.

Shelf Life.

Period of validity for UROGEN-Test-5 is 12 months from the date of acceptance by the manufacturer's Quality Control Department provided that the reagent kit is stored at - 20 °C, and all conditions of transportation, storage and operation are observed. A reagent kit with expired shelf life cannot be used.

Shelf life of opened kit components.

12 months if stored at - 20 °C**.**

Shelf life of kit components ready for operation. 1 hour if stored in an ice bath and complied with conditions that prevent components drying and contamination by outside biological material.

13. Disposal

Reagent kits that have become unusable, including shelf life expiration, are subject to disposal in accordance with SanPiN 2.1.7.2790-10 requirements "Sanitary and Epidemiological Requirements for Medical Waste Handling".

According to medical waste classification, the kits belong to Class A (epidemiologically safe waste close in composition to solid household waste). Unused reagents in accordance with paragraph 4.28 of SanPiN 2.1.7.2790-10 "Sanitary and Epidemiological Requirements for Medical Waste Handling" are collected in a single-use labeled packaging of any color (except yellow and red).

Test tubes and materials after the use are disposed in accordance with Methodology Guidelines 287-113 (Methodology Guidelines for Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices).

Liquid components (reagents) are disposed by draining into a sewer with preliminary dilution of a reagent with tap water 1: 100 and removing the remains of packages as industrial or household garbage.

Consumer packaging of UROGEN-Test-5 is subject to mechanical destruction with the removal of residues as industrial or household garbage.

Personnel disposing reagents must comply with the safety rules for conducting a particular method of disposal.

14. Warranty Obligations, Contacts

The manufacturer guarantees that UROGEN-Test-5 reagent kit meets Technical Specifications (TS) requirements subject to compliance with established requirements for transportation, storage and use.

In case of complaints about reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company “TestGene”
(TestGene, LLC),
9 44th Inzhenerny Proezd, office 13, Ulyanovsk 432072
Tel.: +7 (499) 705-03-75
www.testgen.ru

Technical Support Service:
Tel.: +7 927 981 58 81
E-mail: help@testgen.ru

Instruction for Use complies with the requirements of Order of Ministry of Health of Russia dated 09.01.2014 No. 2n, Order of Ministry of Health of Russia dated 19.01.2017 No. 11n, State Standard GOST 51088-2013.