Instructions for use

Reagent kit for detection of chimeric *TMPRSS2-ERG* mRNA and relative expression level of *PCA3* gene by two-step multiplex RT-PCR-RT method "Prosta-Test-2.0"

TS 21.20.23-027-97638376-2020

Version 3 dated 20.06.2022

*Red-marked rules, standards etc. are local. They should be replaced by relevant ones applicable in a given country.

List of abbreviations

Abbreviations and designations used in the instruction:

PC	prostate cancer
PCR	polymerase chain reaction
RT	reverse transcription
DNA	deoxyribonucleic acid
cDNA	complementary DNA
RNA	ribonucleic acid
ICS	internal control sample
NC	negative control sample
PC	positive control sample
SenC	sensitive control sample

Content

Introduction

Prostate cancer (PC) is one of the most common cancers in men. In the initial stages the disease may be asymptomatic or with symptoms that are caused by associated, more common pathologies (chronic prostatitis and benign prostatic hyperplasia). Early diagnosis of prostate cancer allows for timely treatment, which contributes to patients full recovery.

Prosta-Test-2.0 reagent kit detects **target analyte** – *PCA3* gene non-coding mRNA and chimeric *TMPRSS2-ERG* gene mRNA.

Scientific validity

The test system diagnostic effect is based on identification of two molecular genetic markers.

Chimeric *TMPRSS2-ERG* gene results the androgen-regulated *TMPRSS2* gene fusion with oncogenic ETS family of *ERG* transcription factors. *PCA3* gene may have increased level of expression in prostate tissue in cancer. Therefore, the RNA products of these genes may be present in urine and ejaculate of patients with prostate cancer.

Detection of chimeric *TMPRSS2-ERG* mRNA and relative expression level of *PCA3* gene in relation to the mRNA level of the *KLK3* gene in biological samples can be used for early noninvasive prostate cancer diagnostics and it is an additional criterion for initial or repeat prostate biopsy prescription [1,2, 3].

The reagent kit usage area: clinical laboratory diagnostics, oncology.

Indications and contraindications for use

Indications for use: Prosta-Test-2.0 reagent kit is recommended for diagnostics prostate cancer in men over the age of 40 before initial or repeat biopsy prescription. The RNA testing method is a non-invasive procedure, it does not pose any risks for the human health and does not cause any complications.

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[1] Peshkov M.N., Generozov E.V., Kostryukova E.S. The evolution of markers of prostate cancer. // Clinical laboratory diagnostics. - 2016. – No. 61 (3). – P. 132-140.

[2] Sivkov A.V., Efremov G.D., Mihajlenko D.S., Grigor'eva M.V Combination of *PCA3* and *TMPRSS2* markers in the early diagnosis of prostate cancer (literature review). // Experimental and clinical Urology. – 2014. – No. 3. – p. 20.27.

[3] Clinical guidelines "Prostate cancer" (approved by the Ministry of Health of the Russian Federation, 2021).

Contraindications for use: none were identified if used by well-trained personnel and taking into account the intended use.

Population and demographic aspects of the reagent kit usage

Prosta-Test-2.0 reagent kit is recommended for examination of men over 40 years old.

Sterility: the kit is not sterile.

1. Intended use

Intended use:

Prosta-Test-2.0 reagent kit for qualitative detection of chimeric *TMPRSS2-ERG* mRNA and relative expression level of *PCA3* gene by two-step multiplex RT-PCR-RT method — polymerase chain reaction with hybridization-fluorescence detection in a human RNA sample isolated from samples of fresh or fixed in RNA stabilization and preservation solution cell sediment that was obtained by centrifugation or filtration of urine collected without digital rectal examination (DRE) or of post-DRE urine sample taken at any time when examining men over the age of 40 for prostate cancer before the initial or repeat biopsy prescription.

Functional use: obtained results can be used for prostate cancer diagnosis and obtaining an additional criterion for initial or repeat prostate biopsy prescription in men aged 40 years and older.

Reagent kit potential consumers: Reagent kit for research use only.

2. Method principle

Method

Two-step multiplex reverse transcription and subsequent multiplex allele-specific polymerase chain reaction in real time with hybridization-fluorescence detection.

Test sample type

Material for PCR-RT is human RNA samples isolated from fresh urine sediment or urine sediment fixed in RNA stabilization and preservation solution. Sediment is obtained by centrifugation and filtration of urine morning portion sampled without pre-DRE or post-DRE urine sampled at any time.

Detection principle

Detection of chimeric *TMPRSS2-ERG* mRNA and relative expression level of *PCA3* gene is based on the reverse transcription and subsequent polymerase chain reaction in real time and includes three stages:

1) reverse transcription (cDNA isolation);
2) DNA PCR amplification and real-time hybridization-fluorescence detection of amplification products;
3) results interpretation and relative *PCA3* gene expression level evaluation.

RNA extraction from the test material is carried out using the

recommended extraction procedure. Reverse transcription reaction is carried out with the obtained RNA samples in a reaction buffer using primers and MMLV reverse transcriptase.

Multiplex amplification reactions of *PCA3*, *KLK3*, *TMPRSS2-ERG* gene transcripts and sequences of the internal control sample (ICS) are carried out with cDNA samples obtained during reverse transcription in a reaction buffer using specific to these regions DNA primers and Taq-polymerase enzyme (see Section 9).

The amplification reaction mixture includes fluorescent-labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA-target and get destroyed by Taq-polymerase. It leads to fluorescence intensity increasing.

It allows to register specific amplification product accumulation by measuring the fluorescent signal intensity. Fluorescence signal detection is carried out during PCR via a cycler with in real time fluorescence signal detection system. *PCA3* and *TMPRSS2-ERG* amplification products are registered in different tubes in the channel corresponding to FAM/Green fluorophore, *KLK3* amplification products – in the HEX/Yellow channel, ICS amplification products – in the Cy5/Red channel (Table 1).

Table 1 – Multiplexes included in the reagent kit

Multiplex (primer-mix)	**Channel corresponding to a fluorophore**		
	FAM/Green	**Cy5/Red**	**HEX/Yellow**
PCA3+KLK3	*PCA3*	ICS	*KLK3*
T2E+KLK3	*T2E*	ICS	*KLK3*

ICS (internal control sample) allows to evaluate RNA isolation effectiveness and inhibitors possible presence in a sample that may lead to false negative results.

Method limitations

Contamination during DNA isolation or multiplex PCR reaction stages can be a possible reason for obtaining a false negative result. A false positive result can be detected using a negative control sample.

Damage to the package integrity during transportation.

Expired kit usage or kit storage conditions violation.

Storage conditions violation during samples transportation.

Total time of reverse transcription and subsequent PCR procedures is 3 hours depending on the used cycler (excluding sample preparation).

3. The reagent kit components

Configuration forms

The reagent kit comes in two configuration forms:

1. Prosta-Test-2.0-12 for 12 tests is designed for 12 reverse transcription reactions and 14 PCR reactions of each multiplex. It corresponds to 12 test samples, PC and NC samples during a or to 4 test samples, PC and NC samples in each run.

2. Prosta-Test-2.0-24 for 24 tests is designed for 24 reverse transcription reactions and 26 PCR reactions of each multiplex. It corresponds to 24 test samples, PC and NC during a simultaneous installation in a cycler or to 8 test samples, PC and NC samples in each run.

Reagent kit components

Table 2 — Prosta-Test-2.0-12 configuration form components

Reagent	Description	Quantity, volume
ICS	Transparent colorless liquid	1 tube, 120 µl
RT-enzymes	Transparent colorless liquid	1 tube, 6 µl
RT-buffer	Transparent colorless liquid	1 tube, 114 µl
PCR-buffer 5x	Transparent colorless liquid	1 tube, 112 µl
Primer-mix PCA3	Transparent colorless liquid, may have a pink shade	1 tube, 140 µl
Primer-mix T2E	Transparent colorless liquid, may have a pink shade	1 tube, 140 µl
PC	Transparent colorless liquid	1 tube, 48 µl
NC	Transparent colorless liquid	1 tube, 48 µl

NOTE: Operational documentation (instructions for use and quality certificate) is not included in the bill of materials, but is included in the reagent kit delivery scope. To ensure compliance with transportation conditions the reagent kit is placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. The thermal container is put into an individual package with the instructions for use and the quality certificate for every reagent kit batch.

Table 3 — Prosta-Test-2.0-24 configuration form

Reagent	**Description**	**Quantity, volume**
ICS	Transparent colorless liquid	1 tube, 240 µl
RT-enzymes	Transparent colorless liquid	1 tube, 12 µl
RT-buffer	Transparent colorless liquid	1 tube, 228 µl
PCR-buffer 5x	Transparent colorless liquid	1 tube, 208 µl
Primer-mix PCA3	Transparent colorless liquid, may have a pink shade	1 tube, 260 µl
Primer-mix T2E	Transparent colorless liquid, may have a pink shade	1 tube, 260 µl
PC	Transparent colorless liquid	1 tube, 96 µl
NC	Transparent colorless liquid	1 tube, 96 µl

NOTE: Operational documentation (instructions for use and quality certificate) is not included in the bill of materials, but is included in the reagent kit delivery scope. To ensure compliance with transportation conditions the reagent kit is placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. The thermal container is put into an individual package with the instructions for use and the quality certificate for every reagent kit batch.

Internal control sample (ICS) is a ready for use armored RNA with a unique sequence in buffer solution.

Reverse transcription enzymes (RT-enzymes) are ready for use and contain the RevM Revertase with suppressed RNase activity and enhanced thermal stability and RNasin Ribonuclease Inhibitor.

The reverse transcription Buffer (RT-buffer) is ready for use and contains all the necessary components for the RT reaction including dNTP mix and reverse transcription primers**.**

PCR-buffer 5x is ready for use and contains all the main reagents, including a thermostable DNA polymerase with hot start, deoxynucleotide triphosphates, uracil-DNA glycosylase, and an optimized buffer.

Primer-mix PCA3 is ready for use and contains a multiplex mix of primers and probes:

1. Primers and probes for the *PCA3* gene overexpressed in malignant prostate tumor tissues. Detection is carried out in the FAM/Green channel.
2. Primers and probes for the *KLK3* gene encoding the PSA protein and expressed specifically in prostate tissues. Detection is carried out in the HEX/Yellow channel.
3. Primers and probes for the internal control sample (ICS). Detection is carried out in the Cy5/Red channel.

Primer-mix T2E is ready for use and contains a multiplex mix of primers and probes:

1. Primers and probes for *TMPRSS2-ERG* gene expressed in prostate tumor tissues. Detection is carried out in the FAM/Green channel.
2. Primers and probes for the *KLK3* gene encoding the PSA protein and expressed specifically in prostate tissues. Detection is carried out in the HEX/Yellow channel.
3. Primers and probes for the internal control sample (ICS). Detection is carried out in the Cy5/Red channel.

Positive control sample (PC) is a ready for use plasmid vector with synthetic inserts of DNA sequences, complementary ICS RNA and *PCA3*, *KLK3* and *TMPRSS2-ERG* fragments in TE buffer (10 mM Tris, 1 mM EDTA) in 100 000 copies per 1 ml concentration.

Negative control sample (NC) is ready for use and is sterile RNase-free deionized water.

Cp≤35 in the HEX/Yellow channel indicates correct material sampling and its sufficient amount for the reaction. Threshold value in the HEX/Yellow channel is also used to calculate the *PCA3* gene relative expression level.

Reaction in the FAM/Green channel in the corresponding tube indicates *PCA3* and *TMPRSS2-ERG* genes expression.

Cp≤35 in the Cy5/Red channel indicates nucleic acid extraction sufficient efficiency and reverse transcription inhibitors and PCR inhibitors absence. If there is no reaction, the result should be considered unreliable. In that case it is recommended to retest the sample starting from biomaterial collection. The kit contains no substances for medical use, substances of human or animal origin.

4. Reagent kit characteristics

4.1 Technical and functional characteristics

Table 4.

Indicator	Characteristics and standards	Clause in Technical Specification (TS)
1. Technical characteristics		
1.1 Appearance		
1.1.1 Prosta-Test-2.0-12 reagent kit configuration form		
ICS	Transparent colorless liquid	Section 7, clause 7.6
RT-buffer	Transparent colorless liquid	Section 7, clause 7.6
RT-enzymes	Transparent colorless liquid	Section 7, clause 7.6
PCR-buffer 5x	Transparent colorless liquid	Section 7, clause 7.6
Primer-mix PCA3	Transparent colorless liquid, may have a pink shade	Section 7, clause 7.6
Primer-mix T2E	Transparent colorless liquid, may have a pink shade	Section 7, clause 7.6
PC	Transparent colorless liquid	Section 7, clause 7.6
NC	Transparent colorless liquid	Section 7, clause 7.6
1.1.2 Prosta-Test-2.0-24 reagent kit configuration form		
ICS	Transparent colorless liquid	Section 7, clause 7.6
RT-buffer	Transparent colorless liquid	Section 7, clause 7.6
RT-enzymes	Transparent colorless liquid	Section 7, clause 7.6
PCR-buffer 5x	Transparent colorless liquid	Section 7, clause 7.6
Primer-mix PCA3	Transparent colorless liquid, may have a pink shade	Section 7, clause 7.6
Primer-mix T2E	Transparent colorless liquid, may have a pink shade	Section 7, clause 7.6
PC	Transparent colorless liquid	Section 7, clause 7.6
NC	Transparent colorless liquid	Section 7, clause 7.6

1.2. Completeness	According to Clause 1.4 TS 21.20.23-027-97638376-2020	Section 7, clause 7.12
1.3. Marking	According to Clause 4 TS 21.20.23-027-97638376-2020	Section 7, clause 7.12
1.4. Packaging	According to Clause 5 TS 21.20.23-027-97638376-2020	Section 7, clause 7.12
2. Functional characteristics		
2.1. Positive result with PC	Fluorescence signal growth recorded in tubes with PC in the FAM/Green, HEX/Yellow and Cy5/Red channels, Cp≤35.	Section 7, clause 7.8.2
2.2. Reaction with ICS	Fluorescence signal growth recorded in tubes with ICS in the Cy5 channel, Cp≤35.	Section 7, clause 7.8.2
2.3. Negative result with NC	Fluorescence signal growth recorded in tubes with NC in the FAM/Green, HEX/Yellow and Cy5/Red channels, Cp is absent or Cp>40.	Section 7, clause 7.8.2
2.4. Reaction in tubes with SenC	In tubes with SenC in the FAM, HEX and Cy5 channels in all repetitions (not less than 6) Ct≤40 and standard deviation in the SenC repetitions is not more than 5%.	Section 7, clause 7.8.2
2.5. Reaction in NC+ICS sample	Fluorescence signal growth recorded in tubes with NC+ICS in the Cy5 channel, Cp≤35, and there is no graph in the FAM, HEX channels.	Section 7, clause 7.8.2
2.6. PCA3 gene relative expression level evaluation in PC	PCA3 gene relative expression level in the tube with PC should be within 250≤R≤4000	Section 7, clause 7.8.2
2.7. Assay for correct determination of the PCA3 gene expression relative level	The obtained PCA3 gene relative expression level should correspond to PCA3 gene relative expression level evaluated using the registered comparison kit within 10% tolerance.	Section 7, clause 7.8.2
2.8. Assay for the qualitative detection correctness of the chimeric TMPRSS2-ERG gene	The result obtained by qualitative detection of the TMPRSS2-ERG chimeric gene should correspond with the tissue expression of the TMPRSS2-ERG chimeric gene evaluated using a registered comparison kit.	Section 7, clause 7.8.2

Note: 1) Armored RNA containing synthetic inserts of sequences complementary to the internal control RNA and PCA3, KLK3, and TMPRSS2-ERG genes fragments in a TE-buffer (10 mM Tris, 1 mM EDTA) in 10 copies per 1 µl concentration are used as a SenC (sensitivity control sample) during control PCR.

2) The correctness test for the PCA3 gene relative expression level and the correctness test for the TMPRSS2-ERG chimeric gene qualitative detection were performed during preliminary and technical testings.

4.2 Analytical efficiency characteristics

4.2.1 Analytical specificity

Specific to human *TMPRSS2- ERG, PCA3* and *KLK3* genes transcripts.

TMPRSS2-ERG, *PCA3* and *KLK3* genes target regions analytical specificity was approved *in silico* via the BLAST Resource *(https://blast.ncbi.nlm.nih.gov/Blast.cgi).*

4.2.2 Analytical sensitivity

10 copies per 1 ml of RNA solution

4.2.3 Precision under repeatability conditions

To assess precision under repeatability conditions PC, SenC were examined in 10 repetitions.

Repeatability data were obtained within one laboratory for specific equipment and within a specific reagent kit batch.

To evaluate precision under repeatability conditions the sample arithmetic mean, dispersion, standard deviation, and variation index coefficient were calculated based on the data obtained in control samples repetitions.

Study results showed that the variation index under repeatability conditions is not higher than 5%.

4.2.4 Precision under reproducibility conditions

The test-system reproducibility evaluation is carried out similarly to precision under repeatability conditions (Section 4.2.3) but different batches of the reagent kit are used for testing and testings are carried out in different laboratories, by different operators, on different days, via different PCR cyclers (Reproducibility test block 1, Reproducibility test block 2, Reproducibility test block 3, Reproducibility test block 4).

Intra-assay, inter-assay and inter-series reproducibility were observed during precision testing under reproducibility conditions conduction, coefficient of variation did not exceed 3%.

4.3 Clinical efficiency characteristics:

Table 5 – The Prosta-Test-2.0 reagent kit diagnostic characteristics during the PCA3 gene relative expression level determination

Cycler	Multiplex *PCA3*							
	Fresh morning urine cellular sediment samples (1-29)				Morning urine cell sediment samples fixed in RNA stabilization and preservation medium (30-58)			
	Positive samples observations number	**Negative samples observations number**	**Diagnostic sensitivity** with 95% confidence probability	**Diagnostic specificity with 95%** confidence probability	**Positive samples observations number**	**Negative samples observations number**	**Diagnostic sensitivity** with 95% confidence probability	**Diagnostic specificity** with 95% confidence probability
	without pre-DRE							
DTprime	18	38	90.0% (95% CI:68.30%-98.77%)	100% (95% CI:90.75%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)
CFX 96	18	38	90,0% (95% CI:68.30%-98.77%)	100% (95% CI:90.75%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)
Rotor-Gene Q	18	38	90.0% (95% CI:68.30%-98.77%)	100% (95% CI:90.75%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)
QuantStudio 5	18	38	90.0% (95% CI:68.30%-98.77%)	100% (95% CI:90.75%-100%)	22	36	100% (95% CI:84.56%-100%)	100% (95% CI:90.26%-100%)
	after pre-DRE							
DTprime	20	38	100% (95% CI:83.16%-100%)	100% (95% CI:90.7%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)
CFX 96	20	38	100% (95% CI:83.16%-100%)	100% (95% CI:90.7%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)
Rotor-Gene Q	20	38	100% (95% CI:83.16%-100%)	100% (95% CI:90.7%-100%)	22	36	100% (95% CI:84.5%-100%)	100% (95% CI:90.26%-100%)

QuantStudio 5	20	38	100% (95% CI:83.16%-100%)	100% (95% CI:90.7% -100%)	22	36	100% (95% CI:84.5% -100%)	100% (95% CI:90.26%-100%)

Table 6 — Prosta-Test-2.0 reagent kit diagnostic characteristics study results during chimeric TMPRSS2-ERG mRNA detection

Cycler	**Multiplex *TMPRSS2-ERG***							
	Fresh morning urine cellular sediment samples (1-29)				**Morning urine cell sediment samples fixed in RNA stabilization and preservation medium (30-58)**			
	Positive samples observations number	**Negative samples observations number**	**Diagnostic sensitivity** with 95% confidence probability	**Diagnostic specificity** with 95% confidence probability	**Positive samples observations number**	**Negative samples observations number**	**Diagnostic sensitivity** with 95% confidence probability	**Diagnostic specificity** with 95% confidence probability
	without pre-DRE							
DTprime	14	42	87.50% (95% CI:61.65%-98.45%)	100% (95% CI:91.59% -100%)	16	40	88.89% (95% CI:65.2% - 98.62%)	100% (95% CI:91.19%-100%)
CFX 96	14	42	87.50% (95% CI:61.65%-98.45%)	100% (95% CI:91.59% -100%)	16	40	88.89% (95% CI:65.2% -98.62%)	100% (95% CI:91.19%-100%)
Rotor-Gene Q	14	42	87.50% (95% CI:61.65%-98.45%)	100% (95% CI:91.59% -100%)	16	40	88.89% (95% CI:65.2% - 98.62%)	100% (95% CI:91.19%-100%)
QuantStudio 5	14	42	87.50% (95% CI:61.65%-98.45%)	100% (95% CI:91.59% -100%)	16	40	88.89% (95% CI:65.29% -98.62%)	100% (95% CI:91.19%-100%)
	after pre-DRE							
DTprime	16	42	100% (95% CI:79.41%-100%)	100% (95% CI:91.59% -100%)	18	40	100% (95% CI:81.47% -100%)	100% (95% CI:91.19%-100%)
CFX 96	16	42	100% (95% CI:79.41%-100%)	100% (95% CI:91.59% -100%)	18	40	100% (95% CI:81.47% -100%)	100% (95% CI:91.19%-100%)

Rotor-Gene Q	16	42	100% (95% CI:79.41%-100%)	100% (95% CI:91.59%-100%)	18	40	100% (95% CI:81.47%-100%)	100% (95% CI:91.19%-100%)
QuantStudio 5	16	42	100% (95% CI:79.41%-100%)	100% (95% CI:91.59%-100%)	18	40	100% (95% CI:81.47%-100%)	100% (95% CI:91.19%-100%)

5. Risks associated with the reagent kit usage

1. The kit reagents functional properties loss due to transportation, storage or usage under inappropriate conditions;
2. Clinical material contamination with inhibiting substances;
3. RNA test samples reaction mixtures contamination with PC tube contents or with PCR products;
4. Failure to comply with requirements for sample preparation, testing and disposal due to unqualified personnel work;
5. An unusable kit usage (after the expiration date or in case of damaged packaging).

Total residual risk of using the Prosta-Test-2.0 reagent kit for detection of chimeric *TMPRSS2-ERG* mRNA and relative expression level of *PCA3* gene by two-step multiplex RT-PCR-RT method manufactured by TestGene LLC is acceptable; the benefit of its usage exceeds the risk.

6. Safety precautions

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 No.4n dated June 6, 2012.

All components and reagents included in Prosta-Test-2.0 reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "OSSS. Harmful substances. Classification and general safety requirements".

The reagents included in the Prosta-Test-2.0 reagent kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in Prosta-test-2.0 reagent kit are non-toxic, as they are prepared by mixing separate non-toxic components.

The work should be carried out in a laboratory performing

molecular-biological (PCR) testing of clinical material in accordance with SanPiN 2.1.3684-21 dated 28.01.2021 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow the recommendations laid out in MU 287-113, MU 1.3.2569-09.

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

The following requirements should always be met when working:

remove unused reagents in accordance with SanPiN 2.1.3684-21 dated 28.01.2021 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures";

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

1. use the kit strictly for its intended purpose, according to these instructions;
2. only specially trained personnel are allowed to work with the kit (a specialist with higher medical education who has been trained in licensed qualification courses to conduct PCR diagnostics, as well as 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 necessary precautions are not provided for the magnetic fields effects, external electrical influences, electrostatic discharges, pressure or pressure changes, overloads, or sources of thermal ignition.

The kit contains no substances of human or animal origin with a

potential infectious nature, therefore, precautions against any special, unusual risks during product use or sale are not provided.

7. Required equipment and materials

Work with the Prosta-Test-2.0 reagent kit is carried out in working area 3 (for preparing reactions) (MU 1.3.2569-09).

PCR-RT equipment:

1. PCR cabinet (e.g., BAV-PCR-Laminar-S), Lamsystems, Russia).
2. Vortex (e.g. TETA-2, Biocom, Russia).
3. A set of electronic or automatic variable volume dispensers (e.g. Eppendorf, Germany).
4. Refrigerator for 2°C... 8°C with a freezer for lower than -16°C.
5. Rotary type cycler, e.g., Rotor-Gene Q cycler (Qiagen, Germany) or plate-type cycler, e.g., Real-Time CFX96 Touch (BioRad, USA), DTprime cycler (DNA-Technology LLC, Russia), QuantStudio 5 (Thermo Scientific, USA).

Materials and reagents not included in the kit:

1. Disposable tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. Pipette tip rack (e.g., Axygen, USA) and 0.2 ml microtube rack (e.g., InterLabService, Russia).
3. Lab coat and disposable talc-free gloves;
4. Container with disinfectant;
5. Disposable polypropylene PCR tubes[4]:

a) 0.1 ml (flat cap, not striped), (e.g., Axygen, USA) for placing in a rotor for 72 tubes – for real-time PCR devices with detection through a tube bottom (e.g., Rotor-Gene).

b) 0.2 ml (with domed cap) (e.g., Axygen, USA) – for real-time PCR devices with detection through a cap (e.g., CFX96, DTprime).

6. PCR plates (can be used instead of the tubes specified in Clause 5).
7. Optically transparent film for sealing plates.
8. Sterile saline solution;
9. Mineral oil (to prevent mixture evaporation when used for the reverse transcription reaction in a thermal cycler without a heating lid).
10. NA-Extra reagent kit for DNA/RNA isolation from clinical

[4] Make sure that the PCR tubes are compatible with the used cycler.

material according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated 24.09.2021).

11. STOR-X reagent kit for RNA stabilization in biosamples according to TS 9398-099-46482062-2017, manufactured by DNA-Technology, Russia (registration certificate No. RZN 2018/7775 dated 08.11.2018) (if necessary) (this information needs to be clarified).

8. Test samples

Test sample type

Biological material for the assay is fresh morning urine cellular sediment samples taken without pre-DRE, or urine taken at any time after pre-DRE.

8.1 Clinical material collection procedure

ATTENTION! Before starting work, it is required to study the methodological recommendations "Sampling, Transportation and Storage of Clinical Material for PCR Diagnostics", developed by the FBIS Central Research Institute of Rospotrebnadzor, Moscow, 2012.

Material collection for testing

20-30 ml of fresh morning urine cellular sediment samples taken without pre-DRE, or urine taken at any time after pre-DRE taken into a specific dry sterile 50 ml container.

Conditions for the initial biological material transportation, storage and disposal:

It is recommended to transport and store urine samples and the cellular sediment obtained from it without a solution to stabilize and preserve RNA at +2°C ...+8°C for up to 3 days. Sample preparation procedure should be performed during this period – obtaining urine cellular sediment. The total storage period of a urine sample and cellular sediment obtained from it without a solution for RNA stabilization and preservation should not exceed 3 days at +2°C ... +8°C.

When using solution for RNA stabilization and preservation at +2°C…+8°C — for 10 days.

Samples long-term storage (from 10 days to 1 year) at a temperature lower than -18°C in solution for RNA stabilization and preservation.

ATTENTION! Freezing of urine samples is strictly prohibited.

ATTENTION! Thawing and repeated freezing of urine cellular sediment samples during transportation and storage is undesirable as it can significantly reduce RNA isolation.

Disposal of clinical material (class B) as extremely epidemiologically hazardous waste is carried out in accordance with SanPin 2.1.3684.21.

8.2. Clinical material preparation

It is recommended to follow the urine cellular sediments (RNA isolation material) isolation procedure:

1. Fill sterile, RNase-free labeled plastic centrifuge 50, 15, 10 or 2 ml tubes with urine samples using sterile tips or pipettes;
2. Centrifuge 50, 15, 10 ml tubes at 3000 g for 20 minutes or at 5000 g for 15 minutes; centrifuge 2 ml tubes at 10 000 g for 5 minutes.
3. Not touching the sediments remove supernatant with separate sterile tips or pipettes;
4. When using 15, 10 or 2 ml tubes, carefully layer the next urine portions of the same samples over the sediments with separate sterile tips or pipettes, avoiding cross-contamination, repeat steps 2-4 until the urine volumes are completely used;
5. If it is necessary to store the sediments for more than 3 days, use separate sterile tips to add 1.0 ml of RNA stabilization and preservation solution to the sediments (50-300 µl volume) and gently resuspend the sediments by pipetting;
6. For centrifugation samples in 50, 15, or 10 ml tubes transfer the obtained suspensions into labeled sterile 1.5 or 2 ml plastic tubes with separate sterile tips;
7. Place the tubes in a refrigerator at +2°C...+8°C (do not freeze) or in a freezer at -18°C...-24°C if RNA stabilization and preservation solution has been added.

8.3 RNA isolation from clinical material

(is carried out in the nucleic acid extraction zone)

If solution for RNA stabilization and preservation has been added to the sample, centrifuge the tubes at 10 000 g for 5 minutes and remove the supernatant before RNA isolation. Isolate RNA from the sediment.

ATTENTION! Before the RNA isolation procedure, add 10 µl of internal control sample (ICS) to the studied biomaterial.

To isolate RNA it is recommended to use the following reagent kits:

- NA-Extra reagent kit for DNA/RNA extraction from clinical material according to TS 21.20.23-013-97638376-2019 manufactured by TestGene LLC, Russia (registration certificate: RZN 2021/15428 dated 24.09.2021),

The RNA elution solution recommended minimum volume is 20 µl.

RNA isolation must be performed in strict compliance with the used reagent kit protocol and instructions for use.

Conditions for RNA test samples storage

- at 2°C... 8°C — up to 4 hours.

8.4 Interfering substances and restrictions on the test material use

The potentially interfering substances effect on the Prosta-Test-2.0 reagent kit performance has been examined for potentially interfering substances that may be found during normal use of the reagent kit and that may affect the reagent kit ability to produce reliable results.

Interfering substances may originate from the following external and internal sources:

1) substances used in patient treatment (e.g., medicines);

2) substances found in specific sample types — in this case clinical sample contamination with biological agent (blood hemoglobin) can inhibit a PCR if not sufficiently purified during the RNA isolation;

3) substances added during sample preparation (e.g., medium for RNA stabilization and preservation).

Studied concentrations of interfering substances are shown in Table 7.

Table 7.

Type	Substance	Active component	Concentration
Endogenous	Biological agents	hemoglobin	260 μg/ml
Exogenous	A drug for benign prostatic hyperplasia treatment. 5α-Reductase inhibitor	finasteride	0.001 mg/ml
	A drug for benign prostatic hyperplasia treatment. 5α-Reductase inhibitor	dutasteride	0.0001 mg/ml
	A drug for urinary disorders associated with benign prostatic hyperplasia. Alpha1 blocker	alfuzosin	0.001 mg/ml
	Antitumor agent, Gonadotropin-releasing hormone analog.	leuprorelin	0.00075 mg/ml
	Anti androgen drugs with antitumor activity	bicalutamide	0.03 mg/ml
	Anti androgen drugs with antitumor activity	flutamide	0.05 mg/ml
	Antitumor agent, Gonadotropin-releasing hormone analog.	triptorelin	0.00075 mg/ml
	Antitumor agent, gonadotropin-releasing hormone analog	gozerelin	0.00072 mg/ml

According to the study results, drugs that affect prostate-specific antigen levels, such as finasteride, dutasteride, alphuzosin, and anti-androgen therapy drugs (bicalutamide, flutamide) were classified as PCR inhibitors.

Prosta-Test-2.0 reagent kit cannot be used in patients who take medications that affect the prostate-specific antigen level, such as finasteride, dutasteride, alfuzosin and antiandrogen therapy drugs (bicalutamide, flutamide).

It is necessary to follow the rules for sampling clinical material to reduce the number of PCR inhibitors.

Limitations on test material usage:

- it is not allowed to use test material under storage and transportation conditions violation (temperature, duration, multiple freezing-thawing);

- reliable results are not guaranteed when using urine sediment samples contaminated with hemoglobin (blood),

- it is not allowed to use samples contaminated with extraneous biological material.

- the RNA obtained after the isolation procedure must be used immediately for reverse transcription reaction, as the RNA preparation cannot be stored.

- unreliable results may be obtained due to operator mistakes during clinical sample collection and RNA isolation, violation of the reagent kit instructions for use.

- Prosta-Test-2.0 reagent kit cannot be used in patients taking medications that affect prostate-specific antigen levels, such as finasteride, dutasteride, alphuzosin and antiandrogen therapy drugs (bicalutamide, flutamide);

- prostatectomy, radiotherapy, prostate biopsies, etc. may affect the viability of prostate tissue and thus the PCA3 value.

9. Testing procedure

It is not required to install, adjust and calibrate the reagent kit for commissioning.

ATTENTION! It is required to use only disposable sterile plastic consumables that have a special "RNase-free" label when working with RNA. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

9.1. Reverse transcription reaction

Test tubes preparation for reverse transcription reaction

Type of tubes for reverse transcription reaction depends on the used thermal cycler (cycler). Use disposable tips with a filter for adding reagents and RNA probes into the tubes.

ATTENTION! The reaction mixture components should be mixed right before the reverse transcription reaction. Mix the reagents sufficient for the necessary reactions number according to the instructions.

1. Thaw the RT-buffer completely at room temperature and precipitate droplets from the tube lids before starting the work. Take the RT-enzymes out of the freezer right before use. Do not store at room

temperature for a long time (more than 15 minutes).

2. After thawing mix thoroughly the tubes contents (mix using vortex for several seconds), remove the drops by short centrifugation.

3. Take the sufficient tubes number for reverse transcription corresponding to the number of test samples. Type of tubes, rube strips or tube sets for reverse transcription reaction depends on the used thermal cycler (cycler).

4. It is recommended to hold 1.5 or 0.2 ml plastic tubes prepared for reverse transcription under UV light for 20 minutes in a laminar or PCR cabinet where the reactions will be prepared.

Reverse transcription reaction

(is carried out in the PCR area)

The substrate for the reverse transcription reaction is RNA isolated according to Section 8.3.

During the reverse transcription reactions preparation, it should be remembered that RNA degrades rapidly, so all manipulations should be carried out without delay and without long pauses between stages. Start preparing the reverse transcription reaction mixture only after the RNA isolation procedure is complete.

Total reverse transcription reaction volume is 20 µl.

ATTENTION! It is forbidden to change the reaction volume. If the volume is changed, the method sensitivity decreases dramatically!!!

1. Add 9.5 µl of PCR buffer into each tube.[5]
2. Add 0.5 µl of RT-enzymes into each tube[4].
3. Transfer 10 µl of isolated RNA into separate tubes with separate tips, mix by pipetting.
4. Mix thoroughly the tubes content using vortex, remove the drops by short centrifugation.
5. When using a thermal cycler without a heating lid, cover the contents of the tubes with mineral oil to prevent the mixture from evaporating.

[5] When testing 2 or more samples, it is allowed to first prepare the reverse transcription mastermix by mixing 9.5 µl of RT-buffer and 0.5 µl of RT-enzymes for each sample. Mix thoroughly the tubes content using vortex, remove the drops by short centrifugation. Add the prepared mastermix into clean tubes corresponding to the thermal cycler (cycler) used for reverse transcription.

6. Place the test tubes into a thermal cycler at 55°C for 90 minutes.

7. After 90 minutes set the temperature at 80°C and keep for 10 minutes.[6]

8. If necessary, store the reverse transcription mixture for up to 1 day at +4°C.[5]

9.3. PCR testing procedure

Test tubes preparation for amplification.

Chose the tubes type according to the used cycler. Use disposable tips with filters for adding reagents, cDNA and control samples into the tubes.

ATTENTION! Mix the reaction mixture components right before the testing procedure. Mix the reagents according to the required number of reactions including test and control samples according to Table 8.

1. Before starting work completely thaw the reagent kit components for amplification (Primer-mixes, NC, PC, PCR-buffer 5x) and remove the drops from the test tube lids by short centrifugation.

2. After thawing, thoroughly mix the contents of the tubes (by shaking the tubes on a vortex for a few seconds or turning them over 10 times), remove droplets from the tube lids by short centrifugation.

3. Take the required number of tubes for the test samples and DNA control samples amplification. Table 8 shows the recommended PCR tubes layout scheme. Prepare 2 PCR tubes for each cDNA test sample, prepare other 4 PCR tubes for control samples for each run.

4. It is recommended to hold the prepared plastic PCR tubes under UV light for 20 minutes in a laminar or PCR cabinet where the reactions will be prepared.

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[6] If a cycler is used to perform the reverse transcription reaction, the following amplification program (volume 20 µl) is used:

Temperature, °C	**Duration, min**
55	90
80	10
4	storage

PCR reaction protocol

(is carried out in the PCR area)

Start preparing the PCR reaction mixture only after the reverse transcription reaction is complete.

The total reaction volume is 20 µl.

ATTENTION! It is forbidden to change the reaction volume. If the volume is changed, the method sensitivity decreases dramatically!!!

It is necessary to use a separate tip with an aerosol barrier for each reaction component.

1. It is recommended to prepare reaction mixtures for samples, PC and NC directly in PCR tubes by mixing all the necessary components according to the calculation table 9.[7] For that:

1.1. Place the tubes in 2 horizontal rows, (n+2) tubes in each row (horizontally), where "n" is the number of samples to be tested (see Table 8).

In this case, the horizontal rows will contain reaction mixtures for each of the two genes (*PCA3*, *TMPRSS2- ERG*), one sample for each gene; the vertical rows will contain the cDNA test samples and control samples.

1.2. Add 4 µl of PCR-buffer 5x into all tubes.

1.3. Add 10 µl of PCA3 primer-mix into (n+2) tubes in the upper horizontal row.

1.4. Add 10 µl of T2E primer-mix into (n+2) tubes in the lower horizontal row.

[7] When examining a large number of samples (more than 4), it is allowed to start from preparation of 2 separate reaction mixtures (PCA3 and T2) by mixing the corresponding PCR buffers and primer mixes according to Table 9. Mix the tubes contents using vortex, remove the drops by short centrifugation. Add 14 µl of the prepared reaction mixtures into the corresponding PCR tubes according to Table 8.

Table 8 – PCR tubes layout scheme

Multiplex	**Sample 1**	**Sample n**	**PC**	**NC**
PCA3	○	○	○	○
T2E	○	○	○	○

Table 9 – Mastermix preparation (for one reaction)

Reaction	**Mastermix**		
	Primer-mix PCA3 or T2E, µl	**PCR-buffer 5x, µl**	**cDNA sample, µl**
PCA3	10	4	6
T2E	10	4	6

2. Add 6 µl of NC into the corresponding tubes (see Table 8).
3. Add 6 µl of PC into the corresponding tubes (see Table 4).
4. Add 6 µl of cDNA into the sample tubes (see Table 8).
5. Seal the PCR plate/close the tubes, make sure that all the lids or the film fit tightly.

6. Remove the PCR plate/tubes to collect the reaction mixture on the well bottoms while maintaining the correct orientation of the plate or the tube set.

7. Load the PCR tubes/plate into a reaction module of a real-time PCR device. It is recommended to install the tubes in the center of the thermoblock to ensure that the tubes are pressed evenly by the heating lid.

8. Program the device to perform the corresponding amplification and fluorescence signal detection programs according to the instructions for the used device. PCR protocol is shown in Table 10.

Table 10 – PCR protocol

Stage	Temperature, ˚C	Time, min:sec	Detection channels	Total cycles
1	95	05:00		1
2	94	00:10		50
	65	00:30	FAM/Green, HEX/Yellow, Cy5/Red	

9. Specify the sample numbers and identifiers, mark the tubes location on the thermoblock matrix in accordance with their installation.

10. Make sure that FAM/Green, HEX/Yellow, Cy5/Red detection channels are applied to the optical measurement parameters.

11. Start amplification program with fluorescence detection.

12. At the end of the program, start analyzing the results.

10. Result registration and interpretation

Results registration is carried out via the used PCR device software for PCR-testing with detection in "real-time" mode. The fluorescence signal accumulation curves are analyzed in the FAM/Green (PCA3, T2E), HEX/Yellow (KLK3) and Cy5/Red (ICS) channels. Threshold cycles (Cp) are recorded.

Results interpretation in control samples

PC results are correct if obtained threshold cycles (Cp) of reactions in PC *KLK3*, *PCA3*, *TMPRSS2-ERG* and ICS do not exceed 35. NC results are correct if there is no reaction or Cp >40 in each of the negative control samples (NC *PCA3*, NC *KLK3*, NC *TMPRSS2-ERG* and ICS) (Table 11).

Table 11 — Assay results for negative and positive control samples

Added material	Selected fluorophore		
	FAM/Green (*PCA3*, *T2E*)	HEX/ Yellow (KLK3)	Cy5/Red (ICS)
NC	Cp >40 or absent	Cp >40 or absent	Cp >40 or absent
PC	Cp ≤35	Cp ≤35	Cp ≤35

When obtaining NC values that differ from those mentioned in Table 11, the entire assay batch results are considered unreliable. In this case take special measures to eliminate possible contamination.

If PC values differ from those indicated in Table 11, it is required to repeat amplification of the entire sample batch. If after repeated amplification PC results differ from those indicated in Table 11, the reagents must be replaced.

Results interpretation in test samples

Start results interpretation in studied test samples only after obtaining correct PC and NC results.

If there are graphs for the *KLK3* gene (HEX/Yellow channel in each tube) the material is sampled correctly. When the threshold cycle (Cp) for the *KLK3* gene (HEX/Yellow channel in each tube) for the sample is more 35 and the threshold cycle (Cp) for ICS (Cy5/Red channel in each tube) is less than 35, the result is considered invalid due to poor biomaterial quality. In this case, it is recommended to repeat the assay starting from the patient's biomaterial collection.

The RNA isolation efficiency, the reverse transcription reaction passage and the PCR inhibition absence in a particular tube are judged by the threshold cycle (Cp) in the ICS reaction. In case of simultaneous absence or late (Cp >35) graphs appearance for the *KLK3* gene (HEX/Yellow channel in each tube) and ICS (Cy5/Red channel in each tube), the result is considered invalid due to the low RNA isolation efficiency, reverse transcription reaction, or PCR inhibition. In this case, it is recommended to repeat the assay starting from the patient's biomaterial collection.

If there are *KLK3* gene graphs (HEX/Yellow channel in each tube) with a threshold cycle (Cp) up to 35, calculate the *PCA3* gene relative

expression level according to the formula[8]:

$R = 1000 \cdot (1{,}92^{(CpKLK3 - CpPCA3)})$, where:

R – relative expression level of the *PCA3* gene,

Cp *KLK3* – *KLK3* reaction threshold cycle (Cp),

Cp*PCA3*[9] – *PCA3* reaction threshold cycle (Cp),

1,92 – average reaction efficiency for *KLK3* and *PCA3* genes,

1000 – coefficient introduced to obtain a convenient, non-fractional data format.

For example, Cp values for KLK3 gene turns out to be 28.7; Cp value for PCA3 gene is 33.3. The relative level of PCA3 expression calculation:

$$R = 1000 \cdot (1{,}92^{(28{,}7\ \ 33{,}3)}) = 1000 \cdot (1{,}92^{\ \ 4{,}6}) = 1000 \cdot 0{,}04975 = 49{,}75$$

Results interpretation principles are shown in Tables 12-13.

Table 12 — Results interpretation principle for multiplex PCA3

PCA3 gene relative expression level	Hex/Yellow channel (*KLK3* gene)	Cy5/Red channel (ICS)	Result
R ≥25	Cp ≤35	not considered	High risk of prostate cancer (regardless of the result in *TMPRSS2/ERG* gene)
R < 25	Cp ≤35	not considered	Low risk of prostate cancer
not considered	Cp >35 or absent	Cp ≤35	The result is invalid due to the sample poor quality
not considered	Cp >35 or absent	Cp >35 or absent	Invalid result due to low RNA isolation efficiency, reverse transcription or PCR inhibition

Table 13 — Results interpretation principle for multiplex T2E

Fam/Green channel (*T2E* gene)	Hex/Yellow channel (*KLK3* gene)	Cy5/Red channel (ICS)	Result
Cp ≤ 40	not considered	not considered	High risk of prostate cancer

[8] Bustin, Stephen A., ed. A-Z of Quantitative PCR. La Jolla, CA: International University Line, 2004-2006

[9] If there are no PCA3 gene graphs and Cp for the KLK3 gene (Hex/Yellow channel) is 35 or less, the PCA3 gene relative expression level (R value) is taken as zero.

			(regardless of the result in *PCA3* gene)
Cp > 40 or absent	Cp ≤35	not considered	Low risk of prostate oncology
Cp > 40 or absent	Cp >35 or absent	Cp ≤35	Invalid result due to the sample low quality
Cp > 40 or absent	Cp >35 or absent	Cp >35 or absent	Invalid result due to low RNA isolation efficiency, reverse transcription or PCR inhibition

11. Storage, transportation and usage conditions

Storage

Store the Prosta-Test-2.0 reagent kit in manufacturer's packaging at -16°C...-24°C during the entire shelf-life period.

It is allowed to freeze/thaw the Prosta-Test-2.0 reagent kit up to 10 times.

The reagent kit stored under the regulated conditions violation cannot be used.

Transportation

The Prosta-Test-2.0 reagent kit can be transported by all types of covered vehicles in accordance with the transportation rules applicable for the vehicle type.

Transport the Prosta-Test-2.0 reagent kit at -16°C… -24°C during the entire shelf-life period.

Atmospheric pressure is not subject to control as it does not affect the reagent kit quality.

To ensure compliance with transportation conditions throughout the entire transportation period, the reagent kit should be placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. Ice packs type, volume and their number in a thermal container and the thermal container size varies according to the transportation duration and conditions.

Reagent kits transported under the temperature conditions violation cannot be used.

Shelf life

The Prosta-Test-2.0 reagent kit shelf life is 12 months from the acceptance date by the manufacturer’s Quality Control Department (QCD) under all the transportation, storage and usage conditions. A reagent kit with an expired shelf life cannot be used.

Opened kit components shelf life

12 months from the acceptance date by the manufacturer's Quality Control Department (QCD) if stored at -16°C...-24°C.

Ready for usage kit components shelf life

1 hour under conditions that prevent drying of the components as well as contamination by extraneous biological material.

12. Disposal

Reagent kits that have become unusable including the ones with expired shelf life, are subject to disposal in accordance with SanPiN 2.1.3684-21 requirements "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures".

According to medical waste classification the kits belong to Class A (epidemiologically safe waste, which is similar in composition to solid household waste). Unused reagents are collected in a single-use labeled packaging of any color (except yellow and red) in accordance with Clause 170 SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Used test tubes and materials are disposed in accordance with MU 287-113 (Methodology Guidelines for Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices).

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

The Prosta-Test-2.0 reagent kit consumer packaging is subject to mechanical destruction with the residues removal as industrial or household garbage.

Personnel carrying out the reagent kit destruction must comply with the safety rules for carrying out one or another destruction method.

13. Warranty, contacts

The manufacturer guarantees the Prosta-Test-2.0 reagent kit quality and safety during the shelf-life period in compliance with the product transportation and storage requirements, as well as in compliance with the usage rules.

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

Limited Liability Company TestGene (TestGene, LLC),
9, 44 Inzhenerny Proezd, office 13, Ulyanovsk, 432072, Russian Federation,
Phone number: +7 (499) 705-03-75
www.testgene.com

Technical Support Service:

Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

Designation	Name of the document
GOST ISO 14971-2011	Medical devices. Application of risk management to medical devices.
GOST R 15.309-98	System of product development and launching into manufacture. Test and acceptance of produced goods. Principal positions.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, kits, the test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R 51352-2013	In vitro diagnostic medical devices. Test methods.
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostic medical devices.
GOST R 56894-2016	Summary technical documentation for demonstrating conformity to the essential principles of safety and performance of in vitro diagnostic medical devices.
GOST R ISO 18113-1-2024	Medical devices for in vitro diagnostics. Information provided by the manufacturer (labeling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2024	Medical devices for in vitro diagnostics. Information provided by the manufacturer (labeling). Part 2. In vitro diagnostics reagents for professional use only.

GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling, and information to be supplied. Part 1. Basic requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.
GOST 2.114-2016	Unified system for design documentation. Specifications
GOST 2.104-2006	Unified system for design documentation. Basic inscriptions.
GOST R 1.3-2018	Standardization in the Russian Federation. Register of technical conditions. Rules for the formation, maintenance and receipt of information.