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INSTRUCTION

Kit for qualitative detection of mutations in the BRCA1,2 genes by multiplex real time PCR with detection of melting curves «BRCA1,2-diagnostics»

TS 21.20.23-024-97638376-2020

Version 3 dd. 27/07/2021

Table of contents

Introduction

BRCA1/2 genes are classic tumor suppressor genes; mutation in these genes results in an impaired regulation of the cell cycle, cell differentiation processes and apoptosis, as well as to an increasing chromosomal instability that leads to an increased risk of hereditary cancers development such as breast cancer, pancreas cancer, endometrial cancer, ovarian cancer, melanoma and male breast cancer[1].

Target analyte to be detected with the *Reagent kit «BRCA1,2-diagnostics»* is mutations in *BRCA1* and *BRCA2* genes — *BRCA1* c.5266dupC, *BRCA1* c.181T>G, *BRCA1* c.5251C>T, *BRCA1* c.5161C>T, *BRCA1* c.4035delA, *BRCA1* c.1961delA, *BRCA2* c.3749dupA, *BRCA1* c.4675G>A, *BRCA2* c.961_962insAA, *BRCA2* c.2897_2898del, *BRCA1* c.68_69del, *BRCA1* c.3700_3704del, *BRCA2* c.8754+1G, *BRCA1* c.4689C>G, *BRCA1* c.3756_3759del, and *BRCA2* 6174delT.

Scientific validation for the target analyte is confined in its specificity to the mutations localized in human *BRCA1* and *BRCA2* genes.

BRCA1/2 genes belong to the family of suppressor genes coding the proteins involved in the reparation process of double-stranded breaks in DNA. When mutation appears, the protein function of this genes is lost resulting in impairment of the main reparation mechanism of double-stranded breaks in DNA.

Analysis for detection of the mutations in *BRCA1* and *BRCA2* genes helps to define the most effective treatment tactics both with targeted agents (PARP-inhibitors) and with different chemotherapy regimens, as well as to predict the progress of breast cancer, ovarian cancer, pancreas cancer, and stomach cancer[2].

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[1] Saptarova L.M., Kogina E.N., Khasanshina L.M., Galimov Sh.N. Analysis of mutations in the BRCA1 and BRCA2 genes in experimental breast cancer patients // Kazan Medical Journal. - 2020. - Volume 101. - №3. - p. 342-346.

[2] Imyanitov E.N. General concepts of hereditary tumor syndromes // Practical Oncology. - 2014. – Vol. 15. - No. 3. - p. 101-106.

[3] Imyanitov E.N. General concepts of hereditary tumor syndromes // Practical Oncology. - 2014. – Vol. 15. - No. 3. - p. 101-106.

As opposed to a sporadic cancer, *BRCA1*-associated breast cancer demonstrated a better response to the therapy, up to complete remission. The survival rate was found to be much higher in the patients with hereditary forms of gynecological cancers than in the patients of overall population, regardless of the stage and administered therapy. Five-year survival rate in the patients with hereditary breast cancer is $58.9 \pm 6.3\%$, while it is $39.7 \pm 4.6\%$ with sporadic cancer. Genetic testing is also important because BRCA status may be potentially used as a predictive marker for chemotherapy administration. Impairments in reparation system suggest high efficacy of DNA-damaging agents, such as ionizing radiation and medications. Neoadjuvant therapy with anthracyclines and taxanes demonstrated high efficacy in subjects with the mutations in *BRCA1* and *BRCA2* genes. Cells with impaired mechanisms of the homologous recombination are notable for high sensitivity to platinum-based agents. Based on the several studies results, neoadjuvant therapy with Cisplatin is effective in patients with *BRCA1*-associated breast cancer, vibrant response to the drug independently associated with the triple-negative phenotype and with the mutation in *BRCA1* gene[4].

Selective cell death for *BRCA1/2*-deficient cells was demonstrated with the use of PARP-inhibitors (poly[ADP-ribose]polymerase inhibitors — enzymes that catalyze the process of poly-ADP-ribosylation and participate in DNA reparation). Selective cell death for *BRCA1/2*-deficient cells was demonstrated with the use of PARP inhibitors such as olaparib.

Application area: clinical laboratory diagnostics, oncology.

Indications and contraindications for use

Indications for use: *Reagent kit «BRCA1,2-diagnostics»* is recommended to use for the diagnostics of hereditary cancers (breast cancer, ovarian cancer, pancreas cancer, stomach cancer) in order to define an effective treatment tactics and to predict treatment effectiveness.

Applied DNA testing approach is a non-invasive procedure, it does not pose any risks for the subject health and does not cause any complications.

Contraindications for use were not identified in case the kit is used by specifically trained personnel and according to intended use.

[4] Gelmon K. et al. Targeting triple-negative breast cancer: optimising therapeutic outcomes // Ann Oncol. — 2012. — Vol. 23. — № 9. — P. 2223–2234.

Population and demographic aspects of the medical device use: population and demographic aspects of the *Reagent kit «BRCA1,2-diagnostics»* use were not identified.

Sterility: the product is non-sterile.

1. Intended use

Intended use. *Reagent kit «BRCA1,2-diagnostics»* is intended for qualitative detection of mutations in *BRCA1* gene (c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del), and *BRCA2* gene (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, 6174delT) based on multiplex PCR with melting curves analysis in the sample of human genomic DNA isolated from isolated from clinical material (peripheral blood, buccal scraping) for the diagnostics of hereditary cancers (breast cancer, ovarian cancer, pancreas cancer, stomach cancer) in order to define an effective treatment tactics and to predict treatment effectiveness.

Functional use. Obtained results can be used for the diagnostics of hereditary cancers (breast cancer, ovarian cancer, pancreas cancer, stomach cancer) in order to define an effective treatment tactics and to predict treatment effectiveness.

Qualified prospects of the medical device. The kit is intended for professional use only in medical centers and clinical diagnostic laboratories of oncology profile. The levels of proficiency for qualified potential users are a doctor of clinical diagnostic laboratory or a medical laboratory technician.

2. Methodology principle

Methodology

Real-time multiplex allele specific polymerase chain reaction with melting curves analysis.

Type of test sample

Material for PCR reaction is human genomic DNA sample isolated from clinical material (peripheral blood, buccal scraping).

Determination principle

Detection of mutations in *BRCA1/2* genes — *BRCA1* c.5266dupC, *BRCA1* c.181T>G, *BRCA1* c.5251C>T, *BRCA1* c.5161C>T, *BRCA1* c.4035delA, *BRCA1* c.1961delA, *BRCA2* c.3749dupA, *BRCA1*

c.4675G>A, *BRCA2* c.961_962insAA, *BRCA2* c.2897_2898del, *BRCA1* c.68_69del, *BRCA1* c.3700_3704del, *BRCA2* c.8754+1G, *BRCA1* c.4689C>G, *BRCA1* c.3756_3759del, and *BRCA2* 6174delT, based on multiplex PCR with melting curves analysis in the sample of human genomic DNA isolated from clinical material (peripheral blood, buccal scraping) consists of 3 steps:

1) PCR preparation;
2) PCR-amplification of DNA and real-time hybridization-fluorescent detection with melting curves analysis;
3) interpretation of the results.

Genomic DNA samples are used for amplification reactions of the gene sites in the reaction buffer by means of the primers specific for these DNA sites.

PCR-buffer contains all main reagents including thermostable DNA polymerase, dNTP mix, magnesium ions and optimized buffer.

Primer mix also contains fluorescent-labeled oligonucleotide probes that are hybridized with the complementary site of the amplified target DNA. Upon PCR completion, a step of thermal melting of duplexes begins resulting in fluorescence level change. This change is detected by the device software and presented as diagrams.

The kit contains reagents for multiplex detection of highly specific sites in genomic DNA of *BRCA1/2* genes — *BRCA1* c.5266dupC, *BRCA1* c.181T>G, *BRCA1* c.5251C>T, *BRCA1* c.5161C>T, *BRCA1* c.4035delA, *BRCA1* c.1961delA, *BRCA2* c.3749dupA, *BRCA1* c.4675G>A, *BRCA2* c.961_962insAA, *BRCA2* c.2897_2898del, *BRCA1* c.68_69del, *BRCA1* c.3700_3704del, *BRCA2* c.8754+1G, *BRCA1* c.4689C>G, *BRCA1* c.3756_3759del, and *BRCA2* 6174delT (Table 1).

Table 1 – Contents of the multiplexes in the kit

Multiplex (primer mix)	**Channel conforming to a fluorophore**	
	FAM/ Green	**HEX/ Yellow**
5266/181	*BRCA1* c.5266dupC	*BRCA1* c.181T>G
5251/5161	*BRCA1* c.5251C>T	*BRCA1* c.5161C>T
4035/1961	*BRCA1* c.4035delA	*BRCA1* c.1961delA

3749/4675	*BRCA2* c.3749dupA	*BRCA1* c.4675G>A
961/2897	*BRCA2* c.961_962insAA	*BRCA2* c.2897_2898del
68/3700	*BRCA1* c.68_69del	*BRCA1* c.3700_3704del
8754/4689	*BRCA2* c.8754+1G>A	*BRCA1* c.4689C>G
3756/6174	*BRCA1* c.3756_3759del	*BRCA2* 6174delT

Limitations

False-positive result may be caused by contamination on the step of DNA isolation or on the step of multiplex PCR performance. False-positive result may be detected by using a negative control sample.

Packaging damage in the course of transportation.

The use of the kit with expired shelf life or storage conditions violation.

Storage conditions violation in the course of samples transportation.

Total time of the multiplex PCR procedure is 90 minutes (sample preparation not included).

3. Reagent kit contents

Design options

There is only one design option for this reagent kit supply — *Reagent kit «BRCA1,2-diagnostics»*.

Number of test samples

Each *Reagent kit «BRCA1,2-diagnostics»* contains reagents in amount sufficient for the performance of 48 reactions for each multiplex (5266/181 — *BRCA1* c.5266dupC, *BRCA1* c.181T>G; 5251/5161 — *BRCA1* c.5251C>T, *BRCA1* c.5161C>T; 4035/1961 — *BRCA1* c.4035delA, *BRCA1* c.1961delA; 3749/4675 — *BRCA2* c.3749dupA, *BRCA1* c.4675G>A; 961/2897 — *BRCA2* c.961_962insAA, *BRCA2* c.2897_2898del; 68/3700 — *BRCA1* c.68_69del, *BRCA1* c.3700_3704del; 8754/4689 — *BRCA2* c.8754+1G>A, *BRCA1* c.4689C>G; 3756/6174 — *BRCA1* c.3756_3759del, *BRCA2* 6174delT). This corresponds to the detection procedure for 36 test samples including positive and negative control samples, or to 12 single sets of analysis for test samples with positive and negative control samples in every set.

Kit contents

Table 2 – Contents of the set of supply option for *Reagent kit «BRCA1,2-diagnostics»*

No.	Reagent name	Description	Quantity, volume
1	PCR-buffer	Colorless clear liquid	3 tubes, 1280µl each
2	Primer mix 5266/181	Colorless purple liquid	1 tube 192 µl
3	Primer mix 5251/5161	Colorless purple liquid	1 tube 192 µl
4	Primer mix 4035/1961	Colorless purple liquid	1 tube 192 µl
5	Primer mix 3749/4675	Colorless purple liquid	1 tube 192 µl
6	Primer mix 961/2897	Colorless purple liquid	1 tube 192 µl
7	Primer mix 68/3700	Colorless purple liquid	1 tube 192 µl
8	Primer mix 8754/4689	Colorless purple liquid	1 tube 192 µl
9	Primer mix 3756/6174	Colorless purple liquid	1 tube 192 µl
10	Positive control sample (normal homozygote) - N (PCS-N)	Colorless clear liquid	1 tube, 576 µl
11	Positive control sample (mutant homozygote) - M (PCS-M)	Colorless clear liquid	1 tube, 576 µl
12	Negative control sample (NCS)	Colorless clear liquid	1 tube, 576 µl

PCR-buffer is ready-to-use and contains all main reagents including thermostable DNA polymerase, dNTP mix, magnesium ions and optimized buffer.

Primer mix 5266/181 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA1* c.5266dupC. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA1* c.181T>G. Detection is performed in the channel HEX/Yellow.

Primer mix 5251/5161 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA1* c.5251C>T. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA1* c.5161C>T. Detection is performed in the channel HEX/Yellow.

Primer mix 4035/1961 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA1* c.4035delA. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA1* c.1961delA. Detection is performed in the channel HEX/Yellow.

Primer mix 3749/4675 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA2* c.3749dupA. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA1* c.4675G>A. Detection is performed in the channel HEX/Yellow.

Primer mix 961/2897 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA2* c.961_962insAA. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA2* c.2897_2898del. Detection is performed in the channel HEX/Yellow.

Primer mix 68/3700 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA1* c.68_69del. Detection is performed in the channel FAM/Green.
2. Primers and probe for the mutation detection in *BRCA1* c.3700_3704del. Detection is performed in the channel HEX/Yellow.

Primer mix 8754/4689 is ready-to-use and contains multiplex mix of primers and probes:

1. Primers and probe for the mutation detection in *BRCA2* c.8754+1G>A. Detection is performed in the channel FAM/Green.

2. Primers and probe for the mutation detection in *BRCA1* c.4689C>G. Detection is performed in the channel HEX/Yellow.

Primer mix 3756/6174 is ready-to-use and contains multiplex mix of primers and probes:

3. Primers and probe for the mutation detection in *BRCA1* c.3756_3759del. Detection is performed in the channel FAM/Green.
4. Primers and probe for the mutation detection in *BRCA2* 6174delT. Detection is performed in the channel HEX/Yellow.

Positive control sample (normal homozygote) - N (PCS-N) is a ready-to-use mix of plasmid DNAs with the synthetic inserts of the amplifiable DNA fragments — normal variants of *BRCA1*/2 genes incorporated into plasmid vectors pUC57-BsaI-Free and pAl-TA — with the concentration of 10,000 copies per 1 μl of TE-buffer.

Positive control sample (mutant homozygote) - M (PCS-M) is a ready-to-use mix of plasmid DNAs with the synthetic inserts of the amplifiable DNA fragments — specific fragments with the mutations in *BRCA1* gene (c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del) and *BRCA2* gene (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, 6174delT) incorporated into plasmid vector pAl-TA — with the concentration of 10,000 copies per 1 μl of TE-buffer.

Negative control sample (NCS) is ready-to-use DNase-free deionized water.

The kit does not contain any medicinal products for human use as well as any substances of human or animal origin.

4. Reagent kit characteristics

4.1 Technical and functional characteristics

Table 3 – *Reagent kit «BRCA1,2-diagnostics»*

Parameter	Characteristics and specifications	Article of the Technical Specification (TS)
1. Technical characteristics		**1**
1.1 Appearance		
PCR-buffer	Colorless clear liquid	Section 7, article 7.6
Primer mix 5266/181	Colorless purple liquid	Section 7, article 7.6
Primer mix 5251/5161	Colorless purple liquid	Section 7, article 7.6
Primer mix 4035/1961	Colorless purple liquid	Section 7, article 7.6
Primer mix 3749/4675	Colorless purple liquid	Section 7, article 7.6
Primer mix 961/2897	Colorless purple liquid	Section 7, article 7.6
Primer mix 68/3700	Colorless purple liquid	Section 7, article 7.6
Primer mix 8754/4689	Colorless purple liquid	Section 7, article 7.6
Primer mix 3756/6174	Colorless purple liquid	Section 7, article 7.6
Positive control sample (normal homozygous) – N (PCS-N)	Colorless clear liquid	Section 7, article 7.6
Positive control sample (mutant homozygous) – M (PCS-M)	Colorless clear liquid	Section 7, article 7.6
Negative control sample (NTC)	Colorless clear liquid	Section 7, article 7.6
1.2 Compound	According to point 1.4 by TS 21.20.23-023-97638376-2019	Section 7, article 7.12
1.3 Labeling	According to point 4 by TS 21.20.23-023-97638376-2019	Section 7, article 7.12
1.4 Packaging	According to point 5 by TS 21.20.23-023-97638376-2019	Section 7, article 7.12
2. Functional characteristics		
Positive result with PCS-N	Recording of one melting peak and determination of the melting point	Section 7, article 7.8.3

	on the FAM/Green and HEX/Yellow channels.	
Positive result with PCS-M	Recording of one melting peak and determination of the melting point on the FAM/Green and HEX/Yellow channels.	Section 7, article 7.8.3
Negative result with NCS	There are no melting peaks and melting points on the FAM/Green and HEX/Yellow channels in the tubes with NCS.	Section 7, article 7.8.3

4.2 Analytical performance characteristics

Analytical specificity	Specific to mutations in *BRCA1* gene (c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del) and *BRCA2* gene (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, 6174delT)
Analytical sensitivity	10 copies of *BRCA1, BRCA2* genes in 1 μl of DNA solution

4.3 Clinical performance characteristics

Table 4 – Clinical performance characteristics

Type of test sample material	**Number of samples**	**Number of observations**	**Diagnostic sensitivity** with 90% confidence interval	**Diagnostic specificity** with 90% confidence interval
Peripheral blood	31	62	100% (95% CI: 85.8-100%)	100% (95% CI: 90.7-100%)
Buccal scraping	22	44	100% (95% CI: 83.2-100%)	100% (95% CI: 85.8-100%)

5. List of risks forreagent kit «BRCA1,2-diagnostics»

Borderline risk zone includes:

1. loss of the functional properties of the reagents included in the kit, due to transportation, storage or operation under inappropriate conditions,
2. contamination of the reaction mix containing DNA test sample by the contents of the PC tube or PCR products;

3. performance of the testing procedure with the DNA sample of poor quality (with low concentration and/or poor purification);
4. failure to meet the requirements for sample preparation, testing procedure and disposal because of inappropriate qualification of the personnel;
5. application of the kit unsuitable for use (the use after expiry date or when the packaging was damaged).

No risks have been identified in the unacceptable zone.

Overall residual risk for use of the medical device named «Reagent kit for qualitative detection of *BRCA1/2* genes mutations based on real-time multiplex polymerase chain reaction with melting curves analysis, *Reagent kit «BRCA1,2-diagnostics»*» is acceptable, with the benefit exceeding the risk.

6. Precautions for handling

Depending on the potential risk of use, the product belongs to Class 3 according the nomenclature classification of medical devices approved by the Order of the Ministry of Health of the Russian Federation dd. 06.06.2012 N 4n.

All components and reagents included in the *Reagent kit «BRCA1,2-diagnostics»* contents belong to Class 4 of hazard (low-hazard substances) according to GOST 12.1.007-76 «Occupational safety standards system. Noxious substances. Classification and general safety requirements».

Reagents included in *BRCA1/2*-Diagnostics kit have low vapor pressure, thus the inhalation toxicity is excluded.

Reagents included in *BRCA1/2*-Diagnostics kit are not toxic, as they are prepared by mixing of separate nontoxic components.

Operations with material that is contaminated or suspected for contamination must be performed in compliance with the Sanitary and Epidemiological Regulations (SP) 1.3.3118-13 «Safety in working with microorganisms in pathogenicity (hazard) groups III-IV» and MU 1.3.2569-09 «Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms in pathogenicity groups I-IV».

It is necessary to ensure the personnel work compliance both with the biological safety rules and with the requirements for organization and performance of operations of this kind in order to prevent contamination

of the rooms and equipment by nucleic acids and/or amplicons of the test samples.

Operations must be carried out in a laboratory performing bimolecular (PCR) testing of clinical material in compliance with the requirements of SanPiN 2.1.3684-21 «Sanitary and epidemiological requirements for maintenance of the territories of urban and rural settlements, for water bodies, potable water and potable water supply, air, soils, living quarters, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures». Recommendations of MU 287-113 and MU 1.3.2569-09 must be followed.

The following requirements should always be fulfilled during the work:

- unused reagents, reagents with expired shelf life, as well as used reagents are subject to disposal in compliance with the requirements of SanPiN 2.1.3684-21 «Sanitary and epidemiological requirements for maintenance of the territories of urban and rural settlements, for water bodies, potable water and potable water supply, air, soils, living quarters, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures».

ATTENTION! When discarding waste products after amplification (tubes containing PCR products), it is not allowed to open the tubes and spill their contents because this can result in contamination of the laboratory area, equipment and reagents with PCR products;

1. the kit must be used strictly for its intended use in compliance with this instruction;
2. only specially trained staff (professionals with graduate medical education who completed a certified training course in PCR diagnostics procedures, as well as laboratory technicians who graduated medical school or college [vocational medical training]) is allowed to work with the kit;
3. do not use the kit after the expiry date;
4. avoid contact with skin, eyes and mucous membranes. In case of contact, wash the affected area immediately with water and seek medical attention.

The necessary precautions regarding the effect of magnetic fields, external electric influence, electrostatic discharges, pressure or pressure drops, overload, or sources of thermal ignition are not provided.

This kit does not contain any substances of human or animal origin that have a potential infectious nature. Therefore, no precautions against any specific unusual risks are provided for use or marketing of the product.

7. Required equipment and materials

Manipulations with the *Reagent kit «BRCA1,2-diagnostics»* for multiplex PCR must be performed in working area 3 (zone for preparation of the reactions) (MU 1.3.2569-09).

Equipment for multiplex PCR performance:

1. PCR-cabinet of Class I and Class III biological safety (e.g., BMB-II-Laminar-S-1,2, Laminar Systems, Russia).
2. Vortex (e.g., TETA-2, Biocom, Russia).
3. A set of electronic or automatic pipettes of variable volume (e.g., Eppendorf, Germany).
4. Refrigerator with a temperature from 2 °C to 8 °C, with a freezing chamber (max -16 °C).
5. Thermal cycler[5] with real-time fluorescence hybridization detection in the channels conforming to fluorophores FAM/Green and HEX/Yellow (e.g., CFX96 [BioRad, USA], DT-Prime [DNA-Technology R&D LLC, Russia], Rotor-Gene Q [Qiagen, Germany], QuantStudio 5 [Thermo Fisher Scientific, USA]).

Materials and reagents not included in the kit:

ATTENTION! When working with DNA, it is necessary to use only sterile disposable plastic supplies with a special labeling «DNase-free».

1. Disposable tips with an aerosol barrier of up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA).
2. 1.5 mL disposable Eppendorf tubes.
3. Disposable thin-walled PCR-tubes with optically clear caps:
   - 0.2 mL PCR-tubes;
   - 0.2 mL PCR-tubes strips;
   - PCR-plates with optically clear film (e.g., Axygen, USA).
4. A separate coat and powder free disposable gloves.
5. Container with disinfecting solution.

[5] Thermal cyclers must be maintained, calibrated and operated in compliance with the manufacturer's instructions. The use of this kit in a non-calibrated device may affect the test performance.

6. «Workstation» racks for 0.2 mL tubes or for 0.2 mL tubes strips (e.g., InterLabService, Russia).

8. Test samples

Type of test sample

Material for PCR reaction is human genomic DNA sample isolated from clinical material (peripheral blood, buccal scraping).

8.1 Obtaining of clinical material

ATTENTION! Before any operations, please read the Guidelines for Sampling, transportation and storage of clinical material for PCR-diagnostics developed by the Federal State Institute of Science Central Research Institute of Epidemiology of Federal service for surveillance on consumers' rights protection and human well-being (Rospotrebnadzor), Moscow, 2012.

Test material collection

Peripheral blood.

Collection of the material shall be performed under fasting conditions, or in three hours after meal. Blood shall be drawn from the median cubital vein with a disposable needle (of 0.8-1.1 mm diameter) into a special vacuum collection system (lavender caps — with 6% EDTA solution), or with a disposable syringe into plastic tubes with sodium citrate (3.8% sodium citrate solution, at the ratio of 1:9). The tube shall be closed with a cap, and carefully turned upside down for several times in order to thoroughly mix the blood in the tube with anticoagulant agent (otherwise, blood clotting occurs, and it will be impossible to isolate a DNA).

Do not use heparin as an anticoagulant agent!

Buccal scraping

Collection of the biomaterial shall be performed from the inside lining of the cheek, with a special disposable probe.

Collection of the material shall be performed under fasting conditions, or in 1-1.5 hours after meal. Within one hour before the sample collection procedure, it is also necessary to abstain from drinking anything, smoking and chewing gum.

Ensure the safety stripe is intact, and open the tube with a circular motion.

With a moderate effort, rub the inner lining of one cheek with a swab for 30 seconds (about 25-30 rubbing movements) while swirling the

probe; repeat with the other cheek. After the collection procedure, the swab (applied part of the probe) shall be put into a sterile disposable tube with a snap cap containing appropriate transport medium; the plastic shaft of the probe shall be carefully broken off at the point within 0.5 cm from the applied part — this way, the applied part of the probe with the material is left immersed into transport medium. The cap of the tube shall be closed tightly.

Storage, transportation and disposal conditions of initial clinical material:

Whole blood samples

- at temperatures from 2 °C to 8 °C — up to 24 hours;
- at temperatures from -18 °C to -22 °C — up to 6 months.

Buccal scraping samples

- at room temperature — for 6 hours;
- at temperatures from 2 °C to 8 °C — for 3 days;
- at temperature of -20 °C — for 1 month;
- at temperature of -70 °C — for a long-term storage.

One-time freezing of the material is allowed, if it is impossible to deliver it to the laboratory.

ATTENTION! Repeated cycle of freezing and thawing of the samples must be avoided.

Clinical material (Class B) shall be disposed in compliance with SanPiN 2.1.3684-21, as epidemiologically hazardous waste.

8.2 Obtaining of human genomic DNA sample isolated from clinical material (peripheral blood, buccal scraping)

To isolate a sample of human genomic DNA from the clinical material (peripheral blood, buccal scraping), the following reagent kits are recommended:

- reagent kit intended for nucleic acids isolation RealBest extraction 100, the manufacturer is AO Vector-Best, Russia (Marketing Authorization No. RCF 2010/06867).

or equivalent kits for DNA isolation from the clinical material (peripheral blood, buccal scraping) providing the following quality characteristics of the isolated DNA:

- DNA isolation purity expressed in a an optical dense ratio of A260/280 nm must be not less than 1.7;

- DNA isolation efficiency must be not less than 25%.

DNA isolation procedure must be performed in strict compliance with the protocol and instruction for the reagent kit applied.

Storage conditions of testing DNA samples

- at temperatures from 2 °C to 8 °C — up to 1 day (24 hours);
- at temperatures from -18 °C to -22 °C — up to 1 month;
- at temperature of -80 °C — for a long-term storage.

8.3 Interfering substances and limitations of testing material use

An effect of the potentially interfering substances on the performance of *Reagent kit «BRCA1,2-diagnostics»* was evaluated regarding the potentially interfering substances originating from the following external and internal sources:

1) substances used for the patient treatment (e.g., drugs);

3) substances potentially found in particular types of samples; in this case, clinical sample contamination with blood hemoglobin or mucus (hyaluronic acid) can inhibit a PCR as a result of inappropriate purification in the course of DNA isolation procedure;

3) substances added in the course of sample preparation process (e.g., anticoagulant agents, transport medium).

Table 5 contains test concentrations of the interfering substances expected to occur in the normal course of use of the *Reagent kit «BRCA1,2-diagnostics»*.

Table 5

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 μg/mL
Hyaluronic acid	50 μg/mL
Exogenous interfering substances	
Substances added in the course of sample preparation process	
Heparin (anticoagulant agent)	0.15 mU/mL
Sodium citrate (anticoagulant agent)	0.1 mM/mL
EDTA-K2 (anticoagulant agent)	0.5 mM/mL
Sodium azide (transport medium preservative)	0.075 mmol/mL
Drugs prescribed for oncology diseases therapy	
Ropivacaine (painkiller agent)	0.02 mg/mL

Bevacizumab (agent used for the therapy of colorectal cancer, ovarian cancer, cervical cancer, kidney cancer, glioblastoma, lung cancer, breast cancer)	0.02 mg/mL
Paclitaxel (agent used for the prevention and therapy of non-small-cell lung cancer, breast cancer, ovarian cancer)	0.006 mg/mL
Capecitabine (drug for the therapy of breast cancer, stomach cancer and colorectal cancer)	0.03 mg/mL
Gemcitabine (indicated for use in the therapy of pancreas cancer, lung cancer, bladder cancer)	0.04 mg/mL
Cisplatin (antitumor agent)	0.002 mg/mL

Based on the investigation results, heparin (anticoagulant agent) in the concentration of 0.15 mU/mL is deemed to be a PCR inhibitor in the course of analysis. When peripheral blood is to be drawn, the use of heparin as an anticoagulant agent is not allowed.

It is necessary to comply with the rules of clinical material collection to reduce the amounts of PCR inhibitors.

Limitations for testing material use:

– - Testing material is not suitable for use if storage and transportation conditions were violated (temperature regimen, duration).

– - Samples contaminated with outside biological material are not allowed to use.

– Heparin presence in the blood of patients receiving anticoagulant therapy can lead to invalid results of the PCR performance, so the blood collection for these patients is recommended to be completed before their next drug administration.

9. Testing procedure

Installation, mounting, setup, calibration of the medical product is not required for putting into operation.

ATTENTION! When working with DNA, it is necessary to use only sterile disposable plastic supplies with a special labeling «DNase-free». It is necessary to use a separate tip with an aerosol barrier for each reaction component.

ATTENTION! Reaction mix components should be mixed according to Table 8, immediately before the testing procedure.

9.1 DNA isolation from the biological material

The following kits are recommended for the extraction:

- reagent kit intended for nucleic acids isolation RealBest extraction 100, the manufacturer is AO Vector-Best, Russia (Marketing Authorization No. RCF 2010/06867),

or different equivalent kits intended for nucleic acids isolation from biological material.

DNA isolation procedure must be performed in strict compliance with the protocol and instruction for the reagent kit applied.

Storage conditions of testing DNA samples

- at temperatures from 2 °C to 8 °C — up to 1 day (24 hours);
- at temperatures from -18 °C to -22 °C — up to 1 month;
- at temperature of -80 °C — for a long-term storage.

9.2 PCR performance

Preparation of the components for PCR

Before preparation of the reactions, wet cleaning must be performed for the PCR-cabinet and for the equipment and materials it contains, with the use of disinfectant agents appropriate for application in PCR-laboratories; UV lamp must be turned on for 20-30 minutes.

1. Mix thoroughly the contents of the tubes with the DNA isolated for test, PCR-buffer, primer mix, NTC, PCS-N and PCS-M by turning each tube over for 10 times, or on the vortex with low speed for 3-5 seconds. Then settle the drops from the tube caps by brief centrifugation.

2. Select the number of 0.1-0.2 mL PCR-tubes based on the following calculation for every multiplex to be used: the number of test samples[6] + 1 PCS-N + 1 PCS-M + 1 NTC.

Depending on the purpose to detect specific mutations and the set of supply option in use, every sample shall be set to analysis with one or several multiplexes (primer mixes). Table 5 shows the scheme of the PCR-tubes placement for eight multiplexes to be used.

Table 5 – The scheme of the PCR-tubes placement

Multiplex	Sample 1	Sample n	PC	NC

[6] To increase the accuracy, it recommended to analyze every sample in duplicate.

5266/181	○	○	○	○
5251/5161	○	○	○	○
4035/1961	○	○	○	○
3749/4675	○	○	○	○
961/2897	○	○	○	○
68/3700	○	○	○	○
8754/4689	○	○	○	○
3756/6174	○	○	○	○

To prepare each reaction, the following items are necessary:

1. PCR-buffer — 10 μl;
2. Corresponding primer mix (5266/181, 5251/5161, 4035/1961, 3749/4675, 961/2897, 68/3700, 8754/4689, 3756/6174) — 4 μl;
3. A sample (test sample of DNA[9], PCS-N, PC-M, NTC) — 6 μl.

Total reaction volume — 20 μl.

ATTENTION! Do not change the reaction volume. If the volume is changed, the sensitivity of the method will greatly decrease!

Protocol of the PCR performance

Tubes for reactions must be prepared according to Table 8 in the following order:

1. Label 0.2 mL PCR tubes. For every multiplex, take the number of tubes necessary for test samples + 1 PCS-N +1 PCS-M +1 NTC(Table 5).

2. Aliquot 10 µl of PCR-buffer into each tube[7].

3. Aliquot 4 µl of the primer mix (5266/181, 5251/5161, 4035/1961, 3749/4675, 961/2897, 68/3700, 8754/4689, 3756/6174) into each tube for the corresponding multiplexes (Table 5)[8].

4. Aliquot 6 µl of isolated DNA into the corresponding tubes for the test samples[8]. Do not aliquot the DNA into the tubes for PCS-N, PCS-M and NTC.

6. Aliquot 6 µl of PCS-N and PCS-M into the corresponding tubes for each multiplex to be used.

7. Aliquot 6 µl of NTC into the corresponding tubes for each multiplex to be used.

8. To settle the drops from the tube walls, centrifuge the tubes on the microcentrifuge/vortex for 1-3 seconds.

9. Place the tubes into the reaction module of the real-time PCR machine. It is recommended to place the tubes into the center of the thermal block — this way the tubes will be evenly pressed by a heated lid.

10. Program the machine for the relevant amplification program performance and fluorescent signal detection in compliance with the manual of the machine in use. The PCR protocol is shown in Table 6.

11. Specify the number of the samples and their identifiers, mark the tubes placement on the thermal block matrix according to their pattern of placement.

Table 6 — The PCR protocol

Stage	Temperature, °C	Time, min:sec	Detection channels	Total number of cycles
1	95	02:00	-	1
2	94	00:15		5

[7] It is recommended to prepare the mixture of the primer mix and PCR-buffer first, in a separate 1.5-2.0 mL tube for each multiplex, based on the following calculation: (n + 3) × 4 µL of PCR-buffer + (n + 3) × 10 µL of the corresponding primer mix, with n — the number of samples. Mix on the vortex, settle the drops from the tube caps by brief centrifugation, and aliquot 14 µL into each PCR tube for the corresponding multiplex according to Table 8.

[8] To prevent PCR inhibition, the sample volume may be decreased to 1-5 µL, along with the reaction volume to be adjusted to 20 µL with deionized water from the NTC.

	67	00:30	FAM/Green, HEX/Yellow	
3	94	00:05	-	45
	67	00:30	FAM/Green, HEX/Yellow	
4	25	00:30	-	1
5	25	12:15 AM	FAM/Green, HEX/Yellow, Δt	50 (1,0)

12. Ensure that the detection channels FAM/Green and HEX/Yellow are engaged in the amplification program parameters for optical measurements.

13. Run RT-PCR with melting curves analysis.

14. Upon completion of the program, analyze the results.

10. Recording and interpretation of results

Recording of the results shall be performed upon PCR completion, in a computer-aided manner by means of software of the machine used.

Interpretation of the results shall be performed based on the melting temperature values within the channels FAM/Green and HEX/Yellow (Table 7).

Table 7 — Interpretation of the results within the channels FAM/Green and HEX/Yellow

Multiplex (primer mix)	**Channel conforming to a fluorophore**	
	FAM/ Green	**HEX/ Yellow**
5266/181	*BRCA1* c.5266dupC	*BRCA1* c.181T>G
5251/5161	*BRCA1* c.5251C>T	*BRCA1* c.5161C>T
4035/1961	*BRCA1* c.4035delA	*BRCA1* c.1961delA

3749/4675	*BRCA2* c.3749dupA	*BRCA1* c.4675G>A
961/2897	*BRCA2* c.961_962insAA	*BRCA2* c.2897_2898del
68/3700	*BRCA1* c.68_69del	*BRCA1* c.3700_3704del
8754/4689	*BRCA2* c.8754+1G>A	*BRCA1* c.4689C>G
3756/6174	*BRCA1* c.3756_3759del	*BRCA2* 6174delT

Interpretation of the results in control samples

First, reaction process and melting temperature values shall be evaluated in control samples. In case the process results for PC and NTC are correct, only then the interpretation of the results in test samples may be set up.

The following results must be obtained for the negative and positive control samples (Table 8).

Table 8 – Test results for the negative and positive control samples

Aliquoted material	Selected fluorophore	
	FAM/Green (*BRCA1* c.5266dupC, *BRCA1* c.5251C>T, *BRCA1* c.4035delA, *BRCA2* c.3749dupA, *BRCA2* c.961_962insAA, *BRCA1* c.68_69del, *BRCA2* c.8754+1G>A, *BRCA1* c.3756_3759del)	HEX/Yellow (*BRCA1* c.181T>G, *BRCA1* c.5161C>T, *BRCA1* c.1961delA, *BRCA1* c.4675G>A, *BRCA2* c.2897_2898del, *BRCA1* c.3700_3704del, *BRCA1* c.4689C>G, *BRCA2* 6174delT)
NTC	Absence	Absence
PCS-N	One melting curve peak must be detected and melting temperature must be evaluable	
PCS-M	One melting curve peak must be detected and melting temperature must be evaluable	

The results of the entire analyzed series are considered to be invalid, if the results for the negative control sample are different from the ones specified in Table 8. In this case, special measures must be taken to eliminate possible contamination.

Repeated set of amplification for the entire batch of samples is needed, if the results for the positive control sample are different from the ones specified in Table 8. Reagents must be replaced, if the repeated results for the positive control sample are different from the ones specified in Table 8.

Interpretation of results in test samples

Tables 9-16 demonstrate the interpretive reading principles for the results.

Table 9 – Interpretive reading principles for multiplex 5266/5251 results (mutations detection in *BRCA1* c.5266dupC, *BRCA1* c.181T>G)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA1* c.5266dupC, *BRCA1* c.181T>G are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/c.181T>G — mutation in *BRCA1* c.181T>G is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	c.5266dupC/N — mutation in *BRCA1* c.5266dupC is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 10 – Interpretive reading principles for multiplex 5266/5251 (mutations detection in *BRCA1* c.5251C>T, *BRCA1* c.5161C>T)

Fluorescence channels		Genotype for detection
FAM	HEX	

One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA1* c.5251C>T, *BRCA1* c.5161C>T are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/c.5161C>T — mutation in *BRCA1* c.5161C>T is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	c.5251C>T/N — mutation in *BRCA1* c.5251C>T is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 11 – Interpretive reading principles for multiplex 5266/5251 (mutations detection in *BRCA1* c.4035delA, *BRCA1* c.1961delA)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA1* c.4035delA, *BRCA1* c.1961delA are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/c.1961delA — mutation in *BRCA1* c.1961delA is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	c.4035delA/N — mutation in *BRCA1* c.4035delA is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 12 – Interpretive reading principles for multiplex 3749/4675 (mutations detection in *BRCA2* c.3749dupA, *BRCA1* c.4675G>A)

Fluorescence channels		Genotype for detection
FAM	HEX	

One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA2* c.3749dupA, *BRCA1* c.4675G>A are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/c.4675G>A — mutation in *BRCA1* c.4675G>A is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	c.3749dupA/N — mutation in *BRCA2* c.3749dupA is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 13 – Interpretive reading principles for multiplex 961/470 (mutations detection in *BRCA2 c.961_962insAA, BRCA2 c.2897_2898del*)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA2 c.961_962insAA, BRCA2 c.2897_2898del* are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/*c.2897_2898del* — mutation in *BRCA2 c.2897_2898del* is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	*c. 961_962insAA*/N — mutation in *BRCA2 c. 961_962insAA is detected*
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 14 – Interpretive reading principles for multiplex 68/3700 (mutations detection in *BRCA1 c.68_69del, BRCA1 c.3700_3704del*)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA1 c.68_69del*, *BRCA1 c.3700_3704del* are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/*c.3700_3704del* — mutation in *BRCA1 c.3700_3704del* is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	*c.68_69del*/N — mutation in *BRCA1 c.68_69del* is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 15 – Interpretive reading principles for multiplex 68/3700 (mutations detection in *BRCA2 c.8754+1G>A*, *BRCA1 c.4689C>G*)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA2 c.8754+1G>A, BRCA1 c.4689C>G* are not detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/*c.4689C>G* — mutation in *BRCA1 c.4689C>G* is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	*c.8754+1G>A*/N — mutation in *BRCA2 c.8754+1G>A* is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

Table 16 – Interpretive reading principles for multiplex 3756/6174 (mutations detection in *BRCA1 c.3756_3759del*, *BRCA2 6174delT*)

Fluorescence channels		Genotype for detection
FAM	HEX	
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	N/N — normal homozygous (mutations in *BRCA1 c.3756_3759del, BRCA2 6174delT* are not detected)
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	M/M — mutant homozygous (mutations in *BRCA1 c.3756_3759del, BRCA2 6174delT* are detected)
One melting curve peak different from PCS-N by not more than 2 °C	One melting curve peak different from PCS-M by not more than 2 °C	N/*6174delT* — mutation in *BRCA2 6174delT* is detected
One melting curve peak different from PCS-M by not more than 2 °C	One melting curve peak different from PCS-N by not more than 2 °C	*c.3756_3759del*/N — mutation in *BRCA1 c.3756_3759del* is detected
Melting temperature differs from PCS-N and PCS-M by more than 2 °C		The result is doubtful

Note: N — normal genotype, M — mutant genotype.

To exclude false-negative results, it is recommended to perform repeated PCR with the isolated DNA sample.

Invalid results can be caused by low concentration of DNA, inhibitors present in the DNA sample obtained from the clinical material, deviation from the analysis protocol, violation of the PCR temperature regimen, etc.

In case of invalid or doubtful result, no conclusion is issued; it is necessary to collect biomaterial from the patient again, and repeat the test procedure.

In case of repeated doubtful result, test procedure must be repeated with a reagent kit from a different manufacturer, or with a different method.

11. Storage, transportation and usage conditions

Storage

Kit «BRCA1,2-diagnostics» must be stored at a temperature of minus 16 to minus 24°C in the manufacturer packing during all shelf life. After opening, store in the same conditions as the reagents before opening.

Before use, defrost the PCR buffer at room temperature and mix well by turning the test tube without foam.

Kit stored with violation of storage conditions are not to be applied.

Transportation

Kit «BRCA1,2-diagnostics» must be transported by all kinds of transport at the covered vehicles in accordance with rules of transportation acting on the transport of this type.

Kit «BRCA1,2-diagnostics» must be transported at a temperature of minus 16 to minus 24°C during all shelf life. Transportation at 2 °C to 8 °C is acceptable but no longer than 30 days or at 15°C to 25°C but no longer than 5 days.

Atmosphere pressure is not controlled because it does not influence the sample quality.

For ensuring of transportation conditions during all transportation period the kit is placed into reusable polyurethane-foam thermal container with ice pack for temporary storage and transportation. Type, volume, ice pack amount at transported kits and thermal container volume are selected depending on duration and transportation conditions. Kits transported with violations of temperature conditions are not to be used.

Shelf life

Shelf life of the *Reagent kit «BRCA1,2-diagnostics»* is 12 months from the date of acceptance by the manufacturer's QC under the established transportation, storage and operation conditions. Reagent kit with expired shelf life is not subject to use.

Shelf life of opened kit components

12 months from the date of acceptance by the manufacturer's QC if stored at temperatures from -16 °C to -24 °C.

Shelf life of kit components ready for operation

1 hour if stored under conditions to prevent components drying and contamination by outside biological material.

12. Disposal

Reagent kits that have become unusable for the reasons including but not limited to the expiration of the shelf life, are subject to disposal in compliance with the requirements of SanPiN 2.1.3684-21 «Sanitary and epidemiological requirements for maintenance of the territories of urban and rural settlements, for water bodies, potable water and potable water supply, air, soils, living quarters, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures».

According to the classification of medical waste, the kits belong to Class A (epidemiologically safe waste, which is similar in composition to solid domestic waste). In compliance with article 4.28 of SanPiN 2.1.3684-21 «Sanitary and epidemiological requirements for maintenance of the territories of urban and rural settlements, for water bodies, potable water and potable water supply, air, soils, living quarters, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures», unused reagents must be collected into reusable containers or disposable bags of any color (except of yellow and red).

After performance of the operations, the remaining tubes and materials must be disposed in compliance with MU 287-113 (Guidelines for disinfection, presterilizing cleaning and sterilization of medical devices).

Liquid components (reagents, treating agents) must be disposed by draining into the sewage system, with a preliminary dilution of the reagent with tap water at the ratio of 1:100, and by removal of the remaining packages as industrial or domestic garbage.

Consumer package of *Reagent kit «BRCA1,2-diagnostics»* is subject to mechanical destruction, with removal of residues as industrial or domestic garbage.

Personnel carrying out the destruction of the reagent kit must comply with the safety rules applicable to a particular method of destruction.

13. Warranty obligations, contact information

The manufacturer guarantees the conformity of *Reagent kit «BRCA1,2-diagnostics»* to the TS requirements under transportation, storage and operation conditions established by the technical specification.

If there are any complaints regarding the quality of the kits, undesired events or incidents, please send the information to the following address:

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9, 44th Inzhenerny Proyezd, Ulyanovsk, office 13, 432072, Russia
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