Instructions for use

Reagent kit for qualitative detection of mutations in *BRCA1,2* genes by multiplex real-time polymerase chain reaction with melting curves detection "*BRCA1,2*-tissue"

IVD

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Content

List of abbreviations

Abbreviations and designations used in the instruction:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
NC	negative control sample
PC-N	positive control sample (normal homozygote) - N
PC-M	positive control sample (mutant homozygote) - M

Introduction

BRCA1,2 genes are tumor suppressor genes. Mutations in these genes lead to impaired cell cycle regulation, apoptosis and cell differentiation as well as to increasing chromosomal instability that leads to increased risk of hereditary cancer forms development such as breast cancer, ovarian cancer, pancreatic cancer, stomach cancer[1] [2] [3].

BRCA1,2-tissue reagent kit detects **target analyte** — 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, *BRCA2* 6174delT.

Scientific validity of the target analyte lies in its specificity in relation to mutations in *BRCA1* and *BRCA2* human genes.

The *BRCA1,2* genes belong to suppressor genes that encode proteins involved in double-strand DNA breaks repair. Mutations in these genes lead to proteins function loss, as a result the main mechanism of double-strand DNA break repair gets disrupted.

Assay for mutations detection in the *BRCA1* and *BRCA2* genes helps to determine the most effective treatment strategy with both: targeted drugs (PARP inhibitors) and with various chemotherapy regimens, and also

[1] Clinical Guidelines "Breast cancer", Age group – patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018.

[2] Clinical guidelines "Ovarian cancer/fallopian tube cancer/primary peritoneal cancer", Age group – patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018.

[3] Clinical recommendations "Pancreatic Cancer", Age group – patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018.

makes it possible to predict the course of breast cancer, ovarian cancer, pancreatic cancer, stomach cancer[4].

It was found that *BRCA1*-associated breast cancer has better response to drug therapy comparing to sporadic breast cancer, till the complete remission. It was determined that the survival rate in patients with hereditary gynecologic cancer is significantly higher than in the group of patients without hereditary predisposition, regardless of the stage and treatment: five-year survival rate in patients with hereditary breast cancer is 58,9±6,3% while with sporadic cancer it is 39.7± 4.6%. Genetic testing importance is also determined by the fact that *BRCA* gene status can be potentially used as a predictive marker during chemotherapy treatment. Defects in repair system imply that DNA-damaging agents such as ionizing radiation and drugs are highly effective. High efficacy of neoadjuvant therapy with anthracyclines and taxanes in *BRCA1* and *BRCA2* mutation carriers is shown. Cells with impaired homologous recombination mechanisms are characterized by high sensitivity to platinum derivatives. A number of studies showed that neoadjuvant therapy with Cisplatin is effective for patients with *BRCA1*-associated breast cancer, a significant reaction to the drug is associated with a triple-negative phenotype with mutation in the *BRCA1* gene[5].

According to the studies *BRCA1/2*-deficient tumor cells selectively die if PARP inhibitors are used (PARP (poly (ADP-ribose) polymerase) — enzymes that catalyze poly-ADP-ribosylation; participate in DNA repair). BRCA1/2-deficient cells selectively die when using PARP inhibitors such as Olaparib[6].

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[4] Imyanitov E.N. General ideas about hereditary tumor syndromes // Practical Oncology. 2014. -Vol. 15. - No 3. - P. 101–106.

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

[6] Oza A. M. et al. Olaparib combined with chemotherapy for recurrent platinum sensitive ovarian cancer: a randomized phase 2 trial // Lancet Oncol. — 2015. — Vol. 16.

According to the OVATAR study results conducted in collaboration with the Russian Society of Clinical Oncology (RUSSCO) and a pharmaceutical company AstraZeneca Russia, pathogenic mutations in the *BRCA*1/2 genes are detected in 35% (140/400) of female patients, according to the blood and tumor tissue parallel sequencing results in 400 patients diagnosed with primary ovarian cancer in Russia. At the same time, there are 8 frequent mutations that are officially registered in Russia for a PCR panel, were detected only in 49% of cases (69/140). The other 51% (71/140) of cases were rare pathogenic mutations (occurrence less than 2%) in the *BRCA*1/2 genes by the full gene analysis methods (NGS and MLPA). 30.7% (43/140) of these were rare germline mutations, 15% (21/140) — somatic mutations, and major rearrangements — 5% (7/140).[7]

The scope of the reagent kit is clinical laboratory diagnostics, oncology.

Indications and contraindications for use

Indications for use: *BRCA1,2*–diagnostics reagent kit is recommended for hereditary cancer forms (breast, ovarian, pancreatic and stomach cancer) diagnostics to determine an effective treatment strategy and to predict the treatment effectiveness.

The DNA testing method is a non-invasive procedure, it does not pose any risks for the human health and does not cause any complications.

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:

— № 1. — P. 87–89

[7] Savets V.V. [and others] Final analysis of the OVATAR non-interventional study: diagnostic and therapeutic approaches to the treatment of ovarian cancer in Russia. Analysis of a group with BRCA mutations // Journal of Malignant Tumors. – 2019. – vol. 9. – No.3 S1. – pp. 90-91.

According to the clinical guidelines "Breast cancer", Age group — patients over 18 years old, approved by the Ministry of Health of the Russian Federation, 2018:

"It is recommended to detect the most common germinal mutations in the *BRCA*1/2 genes by polymerase chain reaction in lymphocytes and to consult a geneticist for treatment tactics determination in the following cases:

- in women with confirmed breast cancer with a burdened family history (close relatives ≤50 years old having breast cancer, ovarian or fallopian tube cancer, pancreatic cancer, male breast cancer, metastatic prostate cancer);
- in women with confirmed breast cancer aged <45 years old;
- in women <60 years old with triple negative breast cancer phenotype;
- for premier multifocal breast cancer (including but not limited by diagnosed contralateral breast cancer, ovarian or fallopian tube cancer, pancreatic cancer);
- for breast cancer in men.

Comment: female patients having personal/hereditary history who do not have frequent hereditary mutations should be referred for an extended germline and/or somatic mutation testing using high-throughput sequencing (NGS)."

• According to the clinical guidelines "Ovarian/fallopian tube cancer/primary peritoneal cancer", Age group — patients over 18 years old, approved by the Ministry of Health of the Russian Federation, 2018:

"All female patients with high-grade serous and endometrial carcinomas are recommended to have molecular genetic testing of mutations in the *BRCA*1 and *BRCA*2 genes in blood or oral mucous scraping and/or in biopsy (surgical) material as the disease outcome predictors and for choosing the patient's treatment strategy.

Comment: mutation frequency in the *BRCA*1 and *BRCA*2 genes in the mentioned above tumor types is 15%. Information about the *BRCA* gene mutations is useful for determining whether a tumor is more sensitive to therapy with alkylating drugs, platinum derivatives, and PARP inhibitors."

- According to the clinical guidelines "Pancreatic Cancer", Age group — patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018.

"Molecular genetics testing of mutations in the *BRCA* 1, *BRCA* 2 and *PALB* 2 genes in blood or tumor tissue is recommended for all patients with pancreatic cancer.

Comment: taking into account more than 5% frequency of mutations detection in the *BRCA* 1, *BRCA* 2 and *PALB* 2 genes as well as the significant influence of these mutations on the chemotherapy regimen choice, mutations data determination is recommended for all patients."

Sterility: The kit is not sterile.

1. Intended use

1.1. Intended use: *BRCA1,2* – tissue reagent kit is designed for qualitative detection of mutations in *BRCA1* (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* (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, 6174delT) by multiplex real-time polymerase chain reaction with results detection in the melting curves mode in a human genomic DNA sample extracted from tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) and is used in the examination of patients with tumor diseases hereditary forms (breast cancer, ovarian cancer, pancreatic cancer) in order to determine an effective treatment strategy and predict the treatment effectiveness.

Functional use: obtained results may be used for screening patients with hereditary cancer forms (breast, ovarian, pancreatic and stomach cancer) to determine an effective treatment strategy and to predict treatment effectiveness.

Reagent kit potential consumers:

Reagent kit for research use only.

2. Method principle

Method

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

Test sample type

PCR test material is human genomic DNA samples isolated from 10% formalin-fixed and paraffin-embedded tissue (FFPE-block).

Minimal tumor amount for the assay is 20% according to the results of tumor tissue morphological examination by a histotechnologist.

Method Principle

Mutation detection 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, *BRCA2* 6174delT by multiplex real-time polymerase chain reaction with melting curves detection in human genomic DNA sample extracted from clinical material includes 3 stages:

1. PCR preparation;
2. DNA PCR amplification and real-time hybridization fluorescence detection of amplification products with melting curves detection;
3. Results interpretation.

Gene regions amplification reactions are carried out with DNA samples in a reaction buffer using DNA primers specific to these regions.

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

The primer mix includes fluorescence labeled oligonucleotide probes that hybridize with the amplified target DNA complementary region. Duplexes thermal melting is carried out after PCR completion. It leads to change in fluorescence level that is registered by the device software and is displayed in graphs.

The reagent kit contains reagents for the *BRCA* 1,2 genes genomic DNA highly specific regions multiplex detection — *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, *BRCA2* 6174delT (Table 1).

Table 1 — The reagent kit multiplexes composition

Multiplex (primer mix)	Mutations corresponding to the detection channel	
	FAM	**HEX/ VIC**
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

Method limitations

Minimal tumor amount for analysis is 20% according to the results of tumor tissue morphological examination by a histotechnologist.

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

Damage to the package integrity during transportation.

Using an expired kit or storage conditions violation.

Storage conditions violation during samples transportation.

Total time of the PCR protocol is 2 hours.

3. Reagent kit components

Configuration forms

The reagent kit comes in **one configuration form** — *BRCA1,2*–tissue.

Number of test samples

Each *BRCA1,2*–tissue reagent kit is designed for 48 reactions of 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). It corresponds to 36 test samples including positive and negative control samples or to 12 single runs of test samples with negative and positive control samples in each run.

Reagent kit components

Table 2 — *BRCA1,2*–tissue reagent kit components

No.	Reagent	Description	Quantity, Volume
1	PCR buffer	Transparent colorless liquid	3 tubes, 1280 µl each
2	Primer mix 5266/181	Transparent lilac liquid	1 tube, 192 µl
3	Primer mix 5251/5161	Transparent lilac liquid	1 tube, 192 µl
4	Primer mix 4035/1961	Transparent lilac liquid	1 tube, 192 µl
5	Primer mix 3749/4675	Transparent lilac liquid	1 tube, 192 µl
6	Primer mix 961/2897	Transparent lilac liquid	1 tube, 192 µl
7	Primer mix 68/3700	Transparent lilac liquid	1 tube, 192 µl

8	Primer mix 8754/4689	Transparent lilac liquid	1 tube, 192 µl
9	Primer mix 3756/6174	Transparent lilac liquid	1 tube, 192 µl
10	PC-N	Transparent colorless liquid	1 tube, 576 µl
11	PC-M	Transparent colorless liquid	1 tube, 576 µl
12	NC	Transparent colorless liquid	1 tube, 576 µl

PCR buffer is ready for use and contains all the main reagents, including thermostable DNA polymerase, dNTP mix, and an optimized PCR buffer.

Primer mix 5266/181 is ready for use and contains a multiplex mix of primers and probes:

1. Primers and a probe for a region with a c.5266dupC mutation in BRCA1 gene. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.181T>G mutation. Detection is carried out in the HEX/VIC channel.

Primer mix 5251/5161 is ready for use and contains a multiplex mix of primers and probes:

1. Primers and a probe for a *BRCA1* gene region with c.5251C>T mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.5161C>T mutation. Detection is carried out in the HEX/VIC channel.

Primer mix 4035/1961 is ready for use and contains a multiplex mix of primers and probes:

1. Primers and a probe for a *BRCA1* gene region with c.4035delA mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.1961delA mutation. Detection is carried out in the HEX/VIC channel.

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

1. Primers and a probe for a *BRCA2* gene region with c.3749dupA mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.4675G>A mutation. Detection is carried out in the HEX/VIC channel.

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

1. Primers and a probe for BRCA2 gene region with c.961_962insAA mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA2* gene region c.2897_2898del mutation. Detection is carried out in the HEX/VIC channel.

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

1. Primers and a probe for a *BRCA1* gene region with c.68_69del mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.3700_3704del mutation. Detection is carried out in the HEX/VIC channel.

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

1. Primers and a probe for a *BRCA2* gene region with c.8754+1G>A mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA1* gene region with c.4689C>G mutation. Detection is carried out in the HEX/VIC channel.

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

1. Primers and a probe for a *BRCA1* gene region with c.3756_3759del mutation. Detection is carried out in the FAM channel.
2. Primers and a probe for a *BRCA2* gene region 6174delT mutation. Detection is carried out in the HEX/VIC channel.

Positive control sample — N (PC-N) is a ready for use plasmid DNA mixture with synthetic amplifiable DNA fragment insertions — wild-type *BRCA1*,2 gene variants incorporated into plasmid vectors pUC57-BsaI-Free and pAl-TA with 1000 copies per 1 μl TE-buffer concentration.

Positive control sample — M (PC-M) is a ready for use plasmid DNA mixture with synthetic amplifiable DNA fragment insertions — specific fragments with mutations in *BRCA1* gene (c.5266dupC, c.181T>G, c.5251C>T, c.5161C>T, c.4035delA, c.1961delA, c.4675G>A, c.68_69del, c.3700_3704del, c.4689C>G, c.3756_3759del), *BRCA2* (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, 6174delT) incorporated into pAl-TA plasmid vector with 1000 copies per 1 μl TE-buffer concentration.

Negative control sample (NC) is ready for use DNase-free deionized water.

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

4. The reagent kit characteristics

4.1 Technical and functional characteristics

Table 3 — *BRCA1,2*–tissue reagent kit components

Indicator	Characteristics and standards	Clause in Technical Specification (TS)
Technical characteristics		**1**
1) Description		
PCR buffer	Transparent colorless liquid	Section 7, clause 7.6
Primer mix 5266/181	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 5251/5161	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 4035/1961	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 3749/4675	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 961/2897	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 68/3700	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 8754/4689	Transparent lilac liquid	Section 7, clause 7.6
Primer mix 3756/6174	Transparent lilac liquid	Section 7, clause 7.6
PC-N	Transparent colorless liquid	Section 7, clause 7.6
PC-M	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- 029-97638376-2020	Section 7, clause 7.12
1.3. Marking	According to Clause 1.5 TS 21.20.23- 029-97638376-2020	Section 7, clause 7.12

1.4. Packaging	According to Clause 1.6 TS 21.20.23- 029-97638376-2020	Section 7, clause 7.12
2. Functional characteristics		
Positive result with PC-N	A single melting peak is registered and melting temperature is determined in the FAM and HEX/VIC channels	Section 7, clause 7.8.3
Positive result with PC-M	A single melting peak is registered and melting temperature is determined in the FAM and HEX/VIC channels	Section 7, clause 7.8.3
Negative result with NC	There are no melting peaks and no melting temperature in tubes with NC in the FAM and HEX/VIC channels	Section 7, clause 7.8.3

4.2. Analytical efficiency characteristics

4.2.1 Analytical specificity

Specific to mutations in *BRCA*1 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).

4.2.2 Analytical sensitivity

10 copies of *BRCA*1, *BRCA*2 gene in 1 µl of DNA solution.

4.2.3 The minimum tumor amount for the assay is 20% according to the results of tumor tissue morphological examination by a histotechnologist.

4.2.4 Detection limit (LoD) is the lowest frequency of declared alleles in a sample in the *BRCA*1, *BRCA*2 genes which the reagent kit is able to detect — 5%.

4.2.5 Precision under repeatability conditions

To assess precision under repeatability conditions positive control samples PC-N and PC-M 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 are calculated based on the data obtained in control samples repetitions.

Study results showed that the variation index under repeatability conditions is not higher than 2%.

4.2.6. Precision under reproducibility conditions

The test-system reproducibility evaluation is carried out similarly to precision under repeatability conditions 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, coefficient of variation does not exceed 3% during precision testing under reproducibility conditions conduction.

4.3 Clinical efficiency

53 tissue samples fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) of patients diagnosed with hereditary cancer forms (breast cancer, ovarian cancer, pancreatic cancer) with confirmed positive status of the studied mutations in the *BRCA 1*, *BRCA 2* genes were selected during clinical trials.

Every sample was tested in two repetitions with the *BRCA*1,2-tissue reagent kit manufactured by TestGene LLC for inter-lot repeatability evaluation.

Cyclers recommended by the reagent kit manufacturer that were used for PCR testing:

- DTprime Detecting Cycler (DNA-Technology LLC, Russia);
- CFX 96 (Bio-Rad, USA);
- QuantStudio 5 (Thermo Fisher Scientific, USA).

Results reproducibility for all cyclers is 100%

Table 4 – Clinical efficiency characteristics

Mutation	**Positive samples observation number**	**Negative samples observation number**	**Diagnostic sensitivity** with 95% confidence probability	**Diagnostic specificity** with 95% confidence probability
BRCA1 c.5266dupC	40	66	100% (95% diagnostic interval:91,19%-100%)	(100% (95% diagnostic interval:94,56%-100%)
BRCA1 c.5251C>T	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA1 c.4035delA	8	98	100% (95% diagnostic interval:63,06%-100%)	(100% (95% diagnostic interval:96,31%-100%)
BRCA2 c.3749dupA	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA2 c.961_962insAA	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA1 c.68_69del	2	104	(100% (95% diagnostic interval:15,81%-100%)	(100% (95% diagnostic interval:96,52%-100%)
BRCA2 c.8754+1G>A	2	104	(100% (95% diagnostic interval:15,81%-100%)	(100% (95% diagnostic interval:96,52%-100%)

BRCA1 c.3756_3759del	2	104	(100% (95% diagnostic interval:15,81%-100%)	(100% (95% diagnostic interval:96,52%-100%)
BRCA1 c.181T>G	10	96	(100% (95% diagnostic interval:69,15%-100%)	(100% (95% diagnostic interval:96,23%-100%)
BRCA1 c.5161C>T	8	98	100% (95% diagnostic interval:63,06%-100%)	(100% (95% diagnostic interval:96,31%-100%)
BRCA1 c.1961delA	6	100	(100% (95% diagnostic interval:54,07%-100%)	(100% (95% diagnostic interval:96,38%-100%)
BRCA1 c.4675G>A	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA2 c.2897_2898del	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA1 c.3700_3704del	4	102	(100% (95% diagnostic interval: 39,76%-100%)	(100% (95% diagnostic interval:96,45%-100%)
BRCA1 c.4689C>G	2	104	(100% (95% diagnostic interval:15,81%-100%)	(100% (95% diagnostic interval:96,52%-100%)
BRCA2 6174delT	2	104	(100% (95% diagnostic interval:15,81%-100%)	(100% (95% diagnostic interval:96,52%-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 in concentrations exceeding the permissible ones;
3. Reaction mixtures contamination with test DNA samples contents from a PC tube or with PCR products;
4. Testing with a poor-quality DNA sample (low concentration and/or poor purification);
5. Failure to comply with requirements for sample preparation, testing and disposal due to unqualified personnel work;
6. An unusable kit usage (after the expiration date or in case of damaged packaging).

Total residual risk of using the *BRCA1,2*-tissue reagent kit for qualitative detection of mutations in *BRCA1,2* genes by multiplex real-time polymerase chain reaction with melting curves detection is acceptable; the benefit of its usage exceeds the risk.

6. Safety precautions

All components and reagents included in the *BRCA1,2*-tissue reagent kit belong to low-hazard substances. Precautions against any special, unusual environmental risks when using or selling the product are not provided.

The reagents included in the *BRCA1,2*-tissue reagent kit have low vapor pressure and exclude the inhalation poisoning possibility.

The reagents included in the *BRCA1,2*-tissue 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 clinical material molecular-biological (PCR) testing in accordance with sanitary and epidemiological requirements.

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 sanitary and epidemiological requirements for the management of medical waste.

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 with secondary specialized 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 *BRCA1,2*–tissue reagent kit is carried out in working area 3 (for preparing reactions)

Multiplex PCR equipment:

1. Class II and III biological safety PCR cabinet (e.g. BMB-II-"Laminar-C"-1,2, 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. Cycler with real-time fluorescence detection in the channels corresponding to the FAM, HEX/VIC fluorophores e.g. CFX96 (BioRad, USA), DTprime (NPO DNA Technology LLC, Russia), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the kit:

ATTENTION! It is required to use only disposable sterile plastic consumables that have a special "DNase-free" label when working with a DNA.

1. Disposable tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. 1.5 ml disposable Eppendorf type sterile tubes;
3. PCR plates with an optically transparent film (e.g., Axygen, USA) or thin-walled disposable PCR tubes with an optically transparent lid:
   - 0.2 ml PCR tubes,
   - 0.2 ml PCR tube strips.
4. Lab coat and disposable talc-free gloves;
5. Container with disinfectant;
6. Test tube rack for 0.2ml tubes or for 0.2ml tube strips (e.g., InterLabService, Russia);

7. Reagent kit for DNA extraction from clinical material (see Section 8.2).

8. Test samples

Test sample type

Biological material for the assay is 10% formalin-fixed paraffin-embedded tissue (FFPE-block).

8.1 Clinical material collection procedure

Material collection for testing

Biopsy and/or surgical material.

The material is sampled from a pathological lesion: from its central part and from the part bordering with unchanged tissues. The sampled material is placed in a container with 10% neutral formalin solution. Biological material laboratory processing is performed after fixation, and includes the following procedures — impregnation (dehydration and impregnation with paraffin); embedding in paraffin and paraffin blocks (FFPE-blocks) preparation; microtomy (paraffin sectioning).

Histological preparations suitability criteria for DNA isolation for tumor cells subsequent molecular genetic analysis:

1. According to the morphological analysis results tumor zones should occupy at least 20% of the tissue in a FFPE curl;
2. According to the morphological analysis results hemorrhage and necrosis areas should occupy not more than 15% of the tissue in a FFPE curl;

If the sample does not meet at least one of the listed criteria, it is recommended to use another sample.

When preparing paraffin slides, it is necessary to minimize the samples cross-contamination risk. For that:

- work in disposable talc-free gloves;
- perform the procedure in a PCR cabinet or in a laminar flow cabinet

- use disposable microtome blades and sterile tweezers;
- dispose the first two curls of each block, use curls starting from the third one for molecular research;
- do not place the curls in a water bath.

The initial biological material transportation, storage and disposal conditions:

- at room temperature — during 6 hours;
- at 2°C...8°C — for 3 days;
- at -20°C — for 1 week;
- at -70°C — for a long time.

ATTENTION! Avoid repeated freezing and thawing of samples.

FFPE-blocks transportation and storage conditions:

- at 15°C... 25°C — up to 3 years.

8.2 Human DNA extraction from biological material

To isolate human genomic DNA from biological material it is recommended to use the following reagent kits:

- Reagent kit for human genomic DNA isolation from formalin-fixed and paraffin-embedded tissues (DNA-Tissue-M), manufactured by TestGene LLC, Russia;
- Reagent kit for human genomic DNA isolation from formalin-fixed and paraffin-embedded tissues (DNA-Tissue-F, manufactured by TestGene LLC, Russia.

It is necessary to strictly follow the protocol and the instructions of the used reagent kit during the DNA isolation procedure.

Conditions for DNA test samples possible storage

DNA

- at 2°C... 8°C — up to 24 hours,
- at -18°C... -22°C — up to 1 month,
- at -80°C — for a long time.

Interfering substances and restrictions on the test material use

The potentially interfering substances effect on the *BRCA1,2*-tissue reagent kit performance has been examined for potentially interfering substances that 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 hemoglobin can inhibit a PCR if not sufficiently purified during the DNA isolation;

3) substances added during sample preparation — in this case paraffin, which is used for a FFPE block preparation.

Interfering substances concentrations that are expected to be found during the *BRCA1,2*-tissue reagent kit normal use are listed in Table 5.

Table 5

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 µl/ml
Exogenous interfering substances	
Substances added during sample preparation	
Paraffin	$1*10^{-4}$ µl/µl
Cancer treatment drugs	
Ropivacaine (painkiller)	0.02 mg/ml
Bevacizumab (used for ovarian cancer and breast cancer treatment)	0.02 mg/ml
Paclitaxel (ovarian cancer and breast cancer prevention and treatment)	0.006 mg/ml
Capecitabine (breast cancer treatment drug)	0.03 mg/ml
Gemcitabine (indicated for pancreatic cancer, lung cancer)	0.04 mg/ml

Cisplatin (antitumor agent)	0.002 mg/ml

Based on the study results, potentially interfering substances found during the DNA isolation procedure from clinical material, evaluated at concentrations that are expected to occur during *BRCA1,2*-tissue reagent kit normal use do not affect the test result.

Limitations on test material usage:

- minimum tumor amount for analysis is 20% according to the results of tumor tissue morphological examination by a histotechnologist.
- it is not allowed to use test material under storage and transportation conditions violation (temperature, duration, multiple freezing-thawing);
- it is not allowed to use samples contaminated with extraneous biological material.

9. Testing procedure

Installation, adjustment, calibration of the kit is not required for commissioning.

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

ATTENTION! Reaction mixture components should be mixed right before the assay conduction.

9.1 Kit components preparation for testing

PCR cabinet, equipment and materials contained in it should be wet cleaned using disinfectants suitable for use in PCR laboratories, and exposed to UV-radiation for 20-30 minutes before the reactions preparation.

1. Mix thoroughly the tube contents with the isolated DNA, PCR-buffer, Primer-mixes, NC, PC-N, and PC-M, turn over each tube 10 times or mix using vortex at low speed for 3-5 seconds, then remove the drops from the test tube lids by short centrifugation.

2. Take the required number of 0.1-0.2 ml PCR tubes for each multiplex:

the number of test samples[8] + 1 PC-N + 1 PC-M + 1 NC.

Depending on the necessity to detect specific mutations and on the used reagent kit configuration form each sample should be set to analysis with one or several multiplexes (primer mixes). Table 6 shows the PCR tubes layout scheme for eight multiplexes.

Table 6 – PCR tubes layout scheme

Multiplex	**Sample 1**	**Sample n**	**PC-N**	**PC-M**	**NC**
5266/181	○	○	○	○	○
5251/5161	○	○	○	○	○
4035/1961	○	○	○	○	○
3749/4675	○	○	○	○	○

[8] It is recommended to test each sample in two repetitions to increase accuracy.

961/2897	○	○	○	○	○
68/3700	○	○	○	○	○
8754/4689	○	○	○	○	○
3756/6174	○	○	○	○	○

Each reaction preparation requires:

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. Sample (PC-N, PC-M, NC, DNA test sample[9]) — 6 µl.

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!

9.2 PCR protocol

The reaction tubes should be prepared according to Table 6 in the following order:

1. Label 0.2 ml PCR tubes. For each multiplex take the required tubes number for test samples + 1 PC-N + 1 PC-M + 1 NC (Table 6).
2. Add 10 µl of PCR buffer[9] into each tube.

[9] It is recommended to prepare a mixture of a primer mix and a PCR buffer for each multiplex in a separate 1.5-2.0 ml tube according to the calculation: (n+4)x10 µl of

3. Add 4 µl of primer-mixes (5266/181, 5251/5161, 4035/1961, 3749/4675, 961/2897, 68/3700, 8754/4689, 3756/6174) into the tubes corresponding to multiplexes.

4. Add 6 µl of isolated DNA[10] into the corresponding test samples tubes. Do not add DNA into the tubes for PC-N, PC-M and NC.

5. Add 6 µl of PC-N and PC-M into the corresponding tubes of each used multiplex.

6. Add 6 µl of NC into the corresponding tubes of each used multiplex.

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

8. Install tubes in a PCR-RT device reaction module. It is recommended to install the tubes in the center of a thermoblock to ensure that the tubes are pressed evenly by a heating lid.

9. Program the device to perform the corresponding amplification program and fluorescence detection program according to the instructions for the used cycler. PCR protocol is described in Table 7.

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

PCR buffer + (n+4)x4 µl of the corresponding primer mix, where n is the number of samples. Mix using vortex, remove the drops from the test tube lids by short centrifugation and add 14 µl into PCR tubes for a corresponding multiplex.

[10] To prevent PCR inhibiting a sample volume may be reduced to 1-5 µl, while adjusting the reaction volume to 20 µl with deionized from the NC water.

Table 7 – PCR protocol

Stage	Temperature, ˚C	Time, min:sec	Detection channels	Total cycles number
1	95	02:00	-	1
2	94	00:15	-	5
	67	00:30	-	
3	94	00:10	-	45
	67	00:30	-	
4	95	00:05	-	1
5	25	00:30	-	1
6	25	00:15	FAM, HEX/VIC, Δt	100 (0,5)

11. Make sure that the FAM, HEX/VIC detection channels are applied for the optical measurement parameters of the amplification program.
12. Start PCR with melting curves detection.
13. Start analyzing the results upon the program completion.

10. Results registration and interpretation

Results registration is carried out automatically upon PCR completion with the used device software.

Results interpretation is carried out according to the melting temperature in the FAM, HEX/VIC channels (Table 8).

Table 8 — Results interpretation in the channels

Multiplex (primer mix)	**Mutations corresponding to the detection channel**	
	FAM	**HEX/ VIC**
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

Results interpretation in control samples

First, reaction process and melting temperature in control samples should be evaluated. Start results interpretation in studied test samples only after obtaining correct PC and NC results.

The following results must be obtained for the negative and positive control samples (Table 9).

Table 9 — Test results for a negative and positive control samples

Added material	Selected fluorophore	
	FAM (*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/VIC (*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)
NC	Absent	Absent
PC-N	One melting peak must be detected and melting temperature must be evaluable	
PC-M	One melting peak must be detected and melting temperature must be evaluable	

When obtaining NC values that differ from those mentioned in Table 9, the entire assay batch results are considered unreliable. In this case take special measures to eliminate possible contamination.

If obtained PC values differ from those mentioned in Table 9, it is required to repeat amplification of the entire sample batch. If after repeated amplification obtained PC results differ from those mentioned in Table 9, the reagents must be replaced.

Results interpretation in test samples

Results interpretation principle is shown in Tables 10-17.

ATTENTION! Shown in the tables "trapezoidal graph" indicates a melting curve that has the following characteristics:

- base (the left rising line beginning and the right falling line ending) is almost equal in width to the total width of both PC-N and PC-M bases of the studied multiplex;
- a flat line instead of a peak.

Trapezoid graph (Figure 1) is interpreted as an alternative graph for samples with mutations due to some devices' data processing features.

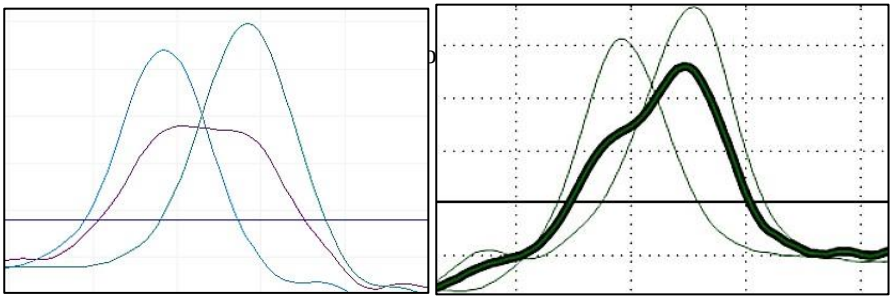

Table 10 — Results interpretation principle for multiplex 5266/181 (*BRCA1* c.5266dupC, *BRCA1* c.181T>G mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td>Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td><td></td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA1 c.5266dupC, BRCA1 c.181T>G mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.181T>G mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than more than 2°C</td><td rowspan="2">BRCA1 c.5266dupC mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C or a trapezoid graph</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 11 — Results interpretation principle for multiplex 5251/5161 (*BRCA1* c.5251C>T, *BRCA1* c.5161C>T mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than more than 2°C</td><td>One melting peak different from PC-N
by not more than 2°C</td><td>N/N — normal homozygote (BRCA1 c.5251C>T, BRCA1 c.5161C>T mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.5161C>T mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C, or a trapezoid graph</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA1 c.5251C>T mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 12 — Results interpretation principle for 4035/1961 multiplex (*BRCA1* c.4035delA, *BRCA1* c.1961delA mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA1 c.4035delA, BRCA1 c.1961delA mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.1961delA mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C, or a trapezoid graph</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA1 c.4035delA mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 13 — Results interpretation principle for 3749/4675 multiplex (*BRCA2* c.3749dupA, *BRCA1* c.4675G>A mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA2 c.3749dupA, BRCA1 c.4675G>A mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.4675G>A mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA2 c.3749dupA mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C, or a trapezoid graph</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 14 — Results interpretation principle for 961/2897 multiplex (*BRCA2 c.961_962insAA, BRCA2 c.2897_2898del* mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA2 c.961_962insAA, BRCA2 c.2897_2898del mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA2 c.2897_2898del mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA2 c.961_962insAA mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 15 — Results interpretation principle for 68/3700 multiplex (*BRCA1* c.68_69del, *BRCA1* c.3700_3704del mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA1 c.68_69del, BRCA1 c.3700_3704del mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.3700_3704del mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA1 c.68_69del mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 16 — Results interpretation principle for 8754/4689 multiplex (*BRCA2* c.8754+1G>A, *BRCA1* c.4689C>G mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA2 c.8754+1G>A, BRCA1 c.4689C>G mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA1 c.4689C>G mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA2 c.8754+1G>A mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

Table 17 — Results interpretation principle for 3756/6174 multiplex (*BRCA1 c.3756_3759del, BRCA2 6174delT* mutations detection)

<table>
<tr><td colspan="2">Fluorescence channels</td><td rowspan="2">Determined genotype</td></tr>
<tr><td>FAM</td><td>HEX</td></tr>
<tr><td>One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-N by not more than 2°C</td><td>N/N — normal homozygote (BRCA1 c.3756_3759del, BRCA2 6174delT mutations are not detected)</td></tr>
<tr><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">BRCA2 6174delT mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td>One melting peak different from PC-M by not more than 2°C</td><td rowspan="2">One melting peak different from PC-N by not more than 2°C</td><td rowspan="2">BRCA1 c.3756_3759del mutation is detected</td></tr>
<tr><td>Two melting peaks different from PC-M and PC-N by not more than 2°C</td></tr>
<tr><td colspan="2">Melting temperature differs from PC-N and PC-M by more than 2°C</td><td>Result is doubtful</td></tr>
</table>

NOTE: "N" - normal genotype, "M" - mutant genotype

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

Reason for obtaining an invalid result may be a low DNA concentration, inhibitors presence in DNA obtained from clinical material; deviation from the assay protocol; the PCR temperature regime violation and etc.

In case of an invalid or doubtful result no conclusion is issued; it is necessary to recollect biomaterial from the patient and repeat the assay.

If a doubtful result repeats, retest with a reagent kit from a different manufacturer or using another method.

11. Storage, transportation and usage conditions

Storage

Store the *BRCA1,2*–tissue reagent kit in manufacturer's packaging at -16°C...-24°C during the entire shelf-life period.

Store the reagents after opening under the same conditions as before opening.

Thaw the PCR buffer at room temperature before use and mix well by turning the tube without foaming.

It is allowed to freeze/thaw the *BRCA1,2*–tissue reagent kit up to 5 times.

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

Transportation

The *BRCA1,2*–tissue reagent kit can be transported by all types of covered vehicles in accordance with the transportation rules applicable for the vehicle type.

Transport the *BRCA1,2*–tissue reagent kit at -16°C… -24°C during the entire shelf-life period. It is allowed to transport the reagent kit at 2°C...8°C up to 30 days or at 15°C...25°C up to 5 days.

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 transportation conditions.

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

Shelf life

The *BRCA1,2*–tissue 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 sanitary and epidemiological requirements for the management of medical waste.

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 sanitary and epidemiological requirements for the management of medical waste.

Used tubes and materials are disposed of in accordance with the requirements 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 *BRCA1,2*–tissue 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 *BRCA1,2*–tissue 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