INSTRUCTIONS FOR USE

Reagent kit for *Plasmodium* spp. DNA quantitative detection "*Plasmodium*-test-Q"

Version 1 dated 11.05.2024

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

1) Name and (or) trade name of a reagent kit

Reagent kit for *Plasmodium* species DNA quantitative detection by PCR-RT *Plasmodium*-test-Q.

Short name ─ *Plasmodium*-test-Q reagent kit.

2) Information on the reagent kit manufacturer

Limited Liability Company TestGene (TestGene LLC),
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Phone number: +7 499 705-03-75
www.testgene.com

3) Intended use

Intended use: *Plasmodium*-test-Q reagent kit is designed for DNA quantitative detection of all clinically significant genus *Plasmodium* representatives, including *P. falciparum, P. vivax, P. malariae, P. ovale curtisi, P. ovale wallikeri* and *P. knowlesi*, without their differentiation, by multiplex polymerase chain reaction with hybridization-fluorescence detection in the real-time (PCR-RT) in clinical samples in order to diagnose malaria in patients with clinical symptoms of the disease and determining the pathogen *Plasmodium* species concentration in the patient's blood.

Functional purpose: the results obtained can be used to support the diagnostics of malaria and the disease treatment.

Target analyte: highly specific regions of the gene encoding 18S rDNA.

The kit is designed for specific disorder, condition or risk factor detection, determination or differentiation: *Plasmodium*-test-Q reagent kit is designed for DNA quantitative detection of clinically significant genus *Plasmodium* representatives, including *P. falciparum, P. vivax, P. malariae, P. ovale curtisi, P. ovale* wallikeri, and *P. knowlesi*, without their differentiation, by multiplex polymerase chain reaction with real-time hybridization-fluorescence detection (PCR-RT) in clinical samples in order to diagnose malaria in patients with clinical symptoms of the disease and determine the pathogen *Plasmodium* spp. concentration in the patient's blood.

The reagent kit for qualitative, semi–quantitative or quantitative detections purpose of use: *Plasmodium*-test-Q reagent kit

is designed for *Plasmodium* spp. DNA quantitative detection in the patient's blood.

Test sample type: DNA obtained from human whole blood.

Indications for use: *Plasmodium*-test-Q reagent kit is recommended for use in patients with clinical symptoms of malaria suspected of infection caused by the genus *Plasmodium* representatives.

Contraindications for use: none.

4) *Plasmodium*-test-Q reagent kit is designed for clinical laboratory testing.

5) Potential consumer of a reagent kit

The kit is intended for research use only.

6) Method principle

Method

Multiplex polymerase chain reaction with real-time hybridization-fluorescence detection (PCR-RT).

Detection principle

The nucleic acids detection of the genus *Plasmodium* representatives is based on a real-time polymerase chain reaction.

5x PCR buffer contains all the basic reagents, including a thermostable hot-start DNA polymerase, deoxynucleotide triphosphates, uracil-DNA glycosidase, and an optimized buffer. The presence of the uracil-DNA glycosidase enzyme prevents false positive results when contaminated with amplification products, while the enzyme is completely inactivated during the first DNA denaturation cycle and does not prevent the current reaction product amplification.

The oligonucleotides mixture contains fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA target and are destroyed by *Taq* polymerase, as a result the fluorescent dye and quencher separate, and fluorescence intensity increases. This allows to register the accumulation of a specific amplification product by real time measuring the fluorescent signal intensity.

The kit contains reagents for quantitative DNA detection of the genus *Plasmodium* representatives, including *P. falciparum, P. vivax, P.*

malariae, P. ovale curtisi, P. ovale wallikeri and *P. knowlesi* (without differentiation), and internal control sample (hereinafter ICS) (Table 1).

Table 1 – Test targets

The channel corresponding to the fluorophore	
FAM / Green	**HEX / Yellow**
18S *Plasmodium* spp.	ICS

Internal control sample (ICS) allows to detect presence of inhibitors in the test sample, the presence of which can lead to false negative results and/or evaluate nucleic acid isolation efficiency.

RT-PCR reaction time ranges from 55 to 75 minutes, (excluding sample preparation) depending on the cycler model used.

7) Reagents, calibrators and control materials description

Configuration forms

The reagent kit is designed in one configuration form: *Plasmodium*-test-Q.

Number of tests

Plasmodium-test-Q reagent kit (Table 2) is designed for 96 reactions, it equates to detection of 90 test samples, negative and positive control samples during a single 96-well cycler run or 13 single test samples detections with negative and positive control samples and calibrators in each run.

Kit components

Table 2 – *Plasmodium*-test-Q reagent kit components

No.	**Reagent name**	**Description**	**Quantity, volume**
1.	5x PCR buffer	Transparent colorless liquid	1 tube, 480 µl
2.	Oligonucleotide mixture	Transparent liquid may have a shade of lilac	1 tube, 480 µl
3.	PC	Transparent colorless liquid	1 tube, 195 µl
4.	NC	Transparent colorless liquid	1 tube, 1300 µl

5.	ICS	Transparent colorless liquid	1 tube, 920 µl
6.	CS1	Transparent colorless liquid	1 tube, 1300 µl
7.	CS2	Transparent colorless liquid	1 tube, 1300 µl

Note: Operational documentation (instructions for use and quality certificate) is not included in the product, but is included in the product delivery set. To ensure compliance with transportation conditions a reagent kit must be placed in a reusable polyurethane foam thermal container with prepared ice packs for temporary storage and transportation. The thermal container, instructions for use and the quality certificate for each batch of products supplied are placed into a cardboard box.

5x PCR buffer is ready for use and contains all the basic reagents, including a thermostable hot start DNA polymerase, deoxynucleotide triphosphates, an uracil-DNA glycosidase enzyme and an optimized buffer. The presence of the uracil-DNA glycosidase enzyme prevents false positive results from contamination with amplification products, while the enzyme is completely inactivated during the first DNA denaturation cycle and does not prevent the current reaction product amplification.

Oligonucleotide mixture is ready for use and contains primers and probes designed to detect specific targets:

1. Primers and probes for *Plasmodium* spp 18S fragment. Detection is carried out in the FAM/Green channel.

2. Primers and a probe for internal control sample. Detection is carried out in the HEX/Yellow channel.

Positive control sample (PC) is ready for use and contains specific fragments of 18S *Plasmodium* spp. detected by the reagent kit, as well as ICS. PC is in a TE buffer (10 mM Tris, 1 mM EDTA) with the addition of 0.05% sodium azide.

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

Internal control sample is ready for use and it is a DNA sample.

Calibration sample No. 1 (CS1) is a DNA sample containing an amplified fragment of *Plasmodium* spp genomic DNA with 1×10^6 IU/ml concentration.

Calibration sample No. 2 (CS2) is a DNA sample containing an amplified fragment of *Plasmodium* spp genomic DNA with 5x10 ^{3} IU/ml concentration.

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

ATTENTION! To perform a reaction, use reagents from the same reagent kit configuration form.

8) Materials and special materials required for testing (assay), but not included in the reagent kit delivery set

Work with *Plasmodium*-test-Q reagent kit is carried out in the working area 3 (for reaction preparation) (MU 1.3.2569-09).

Equipment for multiplex PCR-RT:

1. Class II and III biosafety cabinet (for example, BMB-II-Laminar-C-1.2, Lamsystems, Russia);
2. Vortex (for example, TETA-2, Biocom, Russia);
3. Set of electronic or automatic variable volume dispensers (for example, Eppendorf, Germany);
4. Refrigerator from +2°C to +8°C with freezer lower than -16°C;
5. Cycler[1] with real–time fluorescence detection in channels corresponding to FAM/Green and HEX/Yellow fluorophores - for example, CFX96 (BioRad, USA), DTprime (NPO DNA Technology LLC, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the kit:

ATTENTION! It is required to use only disposable Dnase-free sterile plastic consumables, when working with nucleic acids.

1. Disposable pipette tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (for example, Axygen, USA);
2. 1.5 or 2.0 ml disposable sterile Eppendorf type tubes;
3. Thin-walled disposable tubes with an optically transparent lid (when using plate type cyclers) or optically transparent walls (when using rotary type cyclers) for PCR: 0.1 or 0.2 ml PCR tubes, or 0.1 or 0.2 ml

[1] Cyclers must be maintained, calibrated and used in accordance with the manufacturer's recommendations. The kit usage in an uncalibrated device may affect the performance of the reagent kit.

PCR tube strips, or PCR plates with an optically transparent film (for example, Axygen, USA), compatible with the used cyclers;

4. Lab coat and disposable talc-free gloves;

5. Container with disinfectant solution;

6. Test tube racks for 0.1 or 0.2 ml tubes or for 0.1 or 0.2 ml tube strips (for example, InterLabService LLC, Russia);

7. Racks for 1.5 ml tubes and pipette tips;

8. Magnetic separation rack for 1.5–2.0 ml Eppendorf type tubes;

9. Nucleic acids isolation kit (for example, a reagent kit for DNA/RNA isolation from clinical samples "NA-Extra" according to TS 21.20.23-013-97638376-2019 manufactured by TestGene LLC (registration certificate No. RZN 2021/15428 dated 24.09.2021)

9) Information for reagent kits identification in order to obtain a safe combination and/or information about known limitations on combinations

9.1 To isolate nucleic acids, it is recommended to use reagent kits for isolation from whole blood and ensuring the following quality of the isolated nucleic acids:

- DNA/RNA isolation purity, expressed in terms of optical densities (A260/280) - at least 1.6;
- DNA/RNA isolation efficiency - at least 20%.

9.2 It is required to use only disposable sterile DNase-free plastic consumables when working with DNA.

10) Information on special storage conditions (for example, temperature and humidity, lighting etc.) and/or user's handling of the reagent kit

Storage conditions

Store *Plasmodium*-test-Q reagent kit in the manufacturer's packaging at -18°C… -22°C during the entire kit shelf life. It is allowed to store at +2°C… +6°C up to 30 days. It is allowed to freeze/thaw the kit up to 10 times.

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

11) Information on the reagent kit stability characteristics (for example, storage conditions, shelf life after the primary packaging first opening), as well as solutions storage and stability conditions

Storage

Store *Plasmodium*-test-Q reagent kit in the manufacturer's packaging at -18°C… -22°C during the entire kit shelf life. It is allowed to store at +2°C… +6°C up to 30 days. It is allowed to freeze/thaw the kit up to 10 times.

Store an open kit under the same conditions as before opening.

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

Transportation

Plasmodium-test-Q reagent kit should be transported by all types of covered vehicles in accordance with transportation rules applicable to this transport type.

Transport at -18°C… -22°C during the entire shelf life. Transportation is allowed at +2°C… +6°C up to 30 days or at ambient temperatures lower than +25°C up to 2 days.

Atmospheric pressure is not subject to control, as it does not affect the product quality.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. The type, volume and quantity of ice packs placed in a thermocontainer with the transported reagent kits, as well as the thermocontainer volume depend on the transportation duration and conditions.

Reagent kits transported in the temperature regime violation cannot be used.

Shelf life

Plasmodium-test-Q reagent kit shelf life is 12 months from the acceptance date of the manufacturer's Quality Control Department (QCD) at -18°C… -22°C, if all transportation, storage and operation conditions are met. A reagent kit with an expired shelf life cannot be used.

Opened kit components shelf life is 12 months from the acceptance date of the manufacturer's QCD, if all transportation, storage

and operation conditions are met. A reagent kit with expired shelf life cannot be used.

Ready for usage kit components shelf life – 1 hour, under conditions that prevent the components from drying out, as well as extraneous biological material contamination.

12) Information on sterility, sterilization method and procedure in case of sterile packaging damage

Sterility: the product is not sterile.

13) Information for users (warnings, precautions, necessary measures and limitations when using a reagent kit)

The class, depending on the potential risk of use, is 3, in accordance with the nomenclature classification of medical devices approved by Order of the Ministry of Health of the Russian Federation No. 4n dated 06.06.2012.

All components and reagents included in *Plasmodium*-test-Q reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards system. Harmful substances. Classification and general safety requirements".

The reagents included in *Plasmodium*-test-Q kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in *Plasmodium*-test-Q kit are non-toxic, as they are prepared by mixing separate non-toxic components.

Specialists who have given written consent and have been instructed by employees of Rospotrebnadzor laboratories, which have a sanitary and epidemiological certificate for working with human infectious diseases pathogens of pathogenicity group III, are allowed to work with test systems in the organization laboratory.

Clinical material collection and its packaging is carried out by a medical organization employee trained in the requirements and rules of biological safety when working and collecting material suspected of infection with the pathogenicity group II microorganisms. Each material sample is placed in a separate container for transportation to ensure the requirements of these guidelines.

All samples collected for laboratory testing should be considered

potentially infectious, and medical personnel who collect or transport clinical samples must strictly comply with biosafety requirements, as when working with pathogenicity group III microorganisms.

Samples should be transported in accordance with the requirements of sanitary legislation in relation to pathogenicity group III microorganisms.

All samples obtained for laboratory testing should be considered potentially infected, and the requirements of SP 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases" should be taken into account when working with them. Medical personnel who collect or transport clinical samples to a laboratory should be trained in the safe biomaterial handling, strictly observe the precautions and use personal protective equipment (PPE).

It is necessary to simultaneously ensure and comply with the biological safety rules and requirements for the organization and conduct of these works by personnel in order to prevent premises and equipment contamination with nucleic acids and (or) amplicons of the test samples.

The work should be carried out in a laboratory performing molecular biological (PCR) testing of clinical material in compliance with sanitary and epidemiological rules SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, living quarters, operation of industrial, public facilities, organization and implementation of sanitary-anti-epidemic (preventive) measures". Follow MU 287-113 recommendations.

The following requirements should always be met when working:

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

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

– use the kit strictly for its intended purpose, according to these instructions;

– do not use the kit after the expiration date;

– avoid contact with skin, eyes and mucous membranes. In case of contact, immediately flush the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload or sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with potential infectious nature, therefore, precautions against any special, unusual risks during the product use or sale are not provided.

14) Information on the intended use of a single-use reagent kit

The reagent kit is intended for partial single use.

15) Information on the proper treatment of a reagent kit for reuse, including cleaning, disinfection, packaging and, if necessary, re-sterilization method (if the kit is intended for repeated use)

Not applicable.

16) Special requirements regarding premises, special training or special qualifications of the user and/or third parties

Only specially trained personnel are allowed to work with the kit (a specialist with higher medical education, trained to conduct PCR testing, as well as a laboratory assistant with secondary specialized medical education).

The work should be carried out in a laboratory performing molecular biological (PCR) testing of clinical material in accordance with the applicable rules and regulations.

17) Information on the conditions required for sample collection, treatment and preparation, test samples stability data,

including storage conditions and duration, transportation conditions, limitations on freezing (thawing) cycles

ATTENTION! Before starting work, you should review the guidelines "Sampling, transportation and storage of clinical material for PCR diagnostics" developed by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Test sample type

DNA samples isolated from whole blood.

ATTENTION! Avoid repeated freezing and thawing of samples.

17.1 Clinical material collection

ATTENTION! Clinical material collection, its packaging, labeling and transportation is carried out in accordance with the requirements and rules for handling materials potentially infected with pathogens of pathogenicity group II, their storage and transportation in accordance with MU 1.3.2569-09 "Work organization of laboratories using methods of amplification of nucleic acids when working with material containing microorganisms of pathogenicity groups I–IV".

A medical worker who collects, labels and packs clinical material must be instructed on sanitary and epidemiological requirements and rules of biological safety when working with patients potentially infected with pathogenicity group III microorganisms.

Material sampling for testing

4 or 6 ml peripheral blood is taken in the morning on an empty stomach into a test tube (vacuum tube) containing an EDTA solution. It is not allowed to use heparin as an anticoagulant. Immediately after blood sampling, turn the tube upside down 3-4 times to mix the blood with EDTA.

Transportation and storage conditions of clinical material:

– at +2°C… +8°C – up to 6 hours;

– at room temperature – up to 2 hours.

Hermetically sealed containers with samples are transported to the laboratory in special containers/dressing drums. Referrals and other paper documentation are transferred in a separate plastic bag.

If it is necessary to send samples to the laboratory of another medical institution, fulfill the requirements for the transfer of

pathogenicity group III infectious materials (SP 1.2.036-95 "Procedure for accounting, storage, transfer and transportation of microorganisms of pathogenicity groups I–IV").

Seal test tubes / containers with samples, together with the lid with various sealants (paraffin, parafilm, etc.); label the container. Place the samples of each patient in an individual sealed bag with absorbent material and pack additionally in a common sealed bag.

Two or more samples from the same patient can be packaged in one plastic bag. It is forbidden to pack clinical material samples from different people in the same packaging.

Place the bag with containers in a hermetically sealed container for biological material transportation. Place the container in a foam thermal container with ice packs. Seal and label the transport container. It is recommended to place a disposable indicator that monitors the temperature from +2° to +8°C in the container.

Place the accompanying documents in an individual packaging separately from the biological material and fasten securely to the outside of the container.

17.2. Clinical material preparation [2]

Sample preparation is in accordance with the used kit for nucleic acid isolation.

Add 10 µl of ICS to 100 µl of whole blood plasma during nucleic acids isolation.

Calibration samples CS1 and CS2 in a volume of 100 µl with the addition of 10 µl of ICS as well as NC without ICS addition, also undergo the isolation stage.

If the manufacturer's instructions for the DNA isolation reagent kits allows a larger sample volume use, increase the volume of NC, CS1 and CS2 to the required one with saline solution or TE buffer.

DNA test samples storage conditions: according to the instructions of the isolation reagent kit used.

[2] MU 1.3.2569-09 "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I-IV". Moscow: 2009.

18) Detailed information on the kit reagent preparation for use

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

ATTENTION! It is required to use only disposable DNase-free sterile plastic consumables, when working with nucleic acids. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

ATTENTION! Mix the reaction mixture components immediately before the testing**.**

Reagent kit components preparation for testing

1. Mix thoroughly the tubes contents with nucleic acids isolated for testing, NC, oligonucleotide mixture, 5x PCR buffer, PC, turning each tube 10 times or stirring on a vortex at low speed for 3-5 seconds, and then remove drops from the tube lids by short centrifugation.

2. Take the required number of strips or tubes for the test and control samples amplification.

Before PCR reaction conduction, wet clean the PCR box, as well as the equipment and materials in it, using disinfectants suitable for use in PCR laboratories and turn on the UV lamp for 20-30 minutes.

19) Information necessary for the reagent kit proper installation and readiness for safe operation verification according to the purpose determined by the manufacturer

Not applicable.

20) Recommendations regarding quality control procedures (if needed)

Not applicable.

21) Information on the values traceability for calibrators or control materials, that is provided by reference measurement methods and (or) standards

Metrological traceability of values attributed to calibrators and control materials relative to the reference method: DNA concentration evaluation is carried out by spectrophotometric method during a PC

preparation followed by amplification reaction for metrological traceability of a positive control sample (PC) included in the kit.

22) Testing procedure, including test results calculations and interpretations, and (if needed) information on confirmatory tests advisability

PCR assay consists of the following stages:

A) PCR-RT with nucleic acid samples with hybridization-fluorescence detection of amplification products in real time.

B) Result registration and interpretation.

A) PCR procedure

Kit components preparation

Before preparing reaction mixtures, wet clean the PCR box, as well as the equipment and materials in it, using disinfectants suitable for use in PCR laboratories and turn on the UV lamp for 20-30 minutes.

1. Mix thoroughly the tubes contents with the DNA isolated for testing, 5x PCR buffer, oligonucleotide mixture, NC, CS1, CS2, PC, turning each tube 10 times or stirring on a vortex microcentrifuge at low speed for 3-5 seconds, then remove drops from the tube lids by short centrifugation. NC, CS1 and CS2 samples, that have passed the DNA isolation stage, and add 10 µl of ICS to CS1 and CS2 samples.

2. Take the required number of 0.1 or 0.2 ml tubes (with optically transparent lids or walls, depending on the detecting cycler used type) for PCR based on the following calculation: 1 x test sample number [3]+ 2 x CS1 + 2 x CS + 1 x PC + 1 x NC.

Reaction mixture preparation requires:

1. 5x PCR buffer – 5 µl,
2. Oligonucleotides mixture – 5 µl,
3. Sample (test sample, PC, CS1, CS2 and NC) – 15 µl.

The total reaction volume – 25 µl.

ATTENTION! It is forbidden to change the reaction volume.

[3] To increase accuracy, it is recommended to test each sample twice.

PCR protocol

Prepare the reaction tubes in the following order:

1. Label 0.1 or 0.2 ml tubes for PCR.
2. In a separate 1.5 or 2.0 ml disposable sterile Eppendorf type tube prepare a reaction mixture: (n+8) x5 µl of 5x PCR buffer and (n+8)x5 µl of oligonucleotide mixture, where n is the test sample number. Mix thoroughly the reaction mixture for 3-5 seconds on a vortex microcentrifuge.
3. Add 10 µl of the reaction mixture into the appropriate prepared tubes for PCR.
4. Add 15 µl of the isolated nucleic acids into the appropriate tubes for the test samples. Do not add nucleic acids into the tubes for PC and NC.
5. Add 15 µl of PC into the appropriate tube.
6. Add 15 µl of NC, which has passed the isolation stage, into the appropriate tube, without the ICS addition.
7. Add 15 µl of CS1, which has passed the isolation stage, into the appropriate 2 tubes, with ICS addition.
8. Add 15 µl of CS2, which has passed the isolation stage, into the appropriate 2 tubes, with ICS addition.
9. To remove drops from the walls, centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge.
10. Place the tubes in the real-time PCR device reaction module. It is recommended to place the tubes in the center of the thermoblock to evenly press the tubes with the heating lid.
11. Program the device to perform the corresponding PCR program and detect the fluorescent signal, following the instructions for the device used (Table 3). Testing type: quantitative with standards.

If it is necessary to simultaneously carry out reactions with other reagent kits that require a reverse transcription step, it is acceptable to add the initial stage "52°C – 25 minutes" to the protocol (Table 3), which will not affect the analytical and diagnostic characteristics of the reagent kit.

Table 3 – PCR protocol

Stage	Temperature, °C	Time, min.:sec.	Detection channels	Total number of cycles
1	95	02:00	–	–
2	95	00:15	–	5
	64	00:20		
3	95	00:15	–	45
	64	00:20	FAM/Green, HEX/Yellow	

Specify the number and identifiers of the samples, mark the tubes location on the thermoblock matrix in accordance with their layout.

12. Make sure that the optical measurement parameters of the amplification program include the FAM/Green and HEX/Yellow detection channels.
13. Start PCR with a fluorescent signal detection.
14. Upon the program completion start analyzing the results.

B) Result registration and interpretation.

Results registration is carried out automatically during amplification with the used device software.

Recommendations on setting the threshold line

For cyclers of any model, the threshold line is set individually for each detection channel at a level corresponding to 5-10% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

The result interpretation is performed using the FAM/Green and HEX/Yellow channels Ct values. Only Ct values obtained during the PCR stage with fluorescence detection are taken into account (i.e., corresponding to stage 3 – see Table 3).

The reaction and Ct values in the control samples are evaluated first. Result interpretation in the test samples is carried out only after the correct PC and NC reactions.

If Rotor-Gene 6000, Rotor-Gene 3000, Rotor-Gene Q and similar cyclers are used, activate the functions Dynamic Tube and Noise slope correction, set 10% in Outlier Removal section.

Result interpretation in control samples

The following results should be obtained for negative and positive control samples (Table 4).

Table 4 – PC and NC assay results

Control sample	Selected fluorophore	
	FAM / Green	HEX / Yellow
NC	> 35 or absent	> 35 or absent
PC	Ct ≤ 30	Ct ≤ 32

Note: "absent" – there is no Ct value.

When obtaining NC values that differ from those shown in Table 4, the entire testing series results are considered unreliable. In this case, take special measures to eliminate possible contamination.

When obtaining PC values that differ from those shown in Table 4, it is required to repeat amplification of the entire samples batch. When re-obtaining PC values that differ from those shown in Table 4, the reagents must be replaced.

Result interpretation in DNA test samples

Results interpretation is carried out automatically using the software supplied with the used detection cycler, or manually.

Based on the obtained Ct values for calibration samples and their concentrations (CS1: 10^6 IU/ml; CS2: $5x10^3$ IU/ml), it is necessary to construct a calibration line. For the samples, the values in FAM Ct ≤ 35 are taken into account. When Ct values > 35 are obtained for the samples (if Ct value of ICS ≤ 32), the result is considered doubtful.

PCR efficiency should be in 90-110% range. The difference between the Ct values for repeats of each calibration sample, CS1 and CS2, should be no more than 2. Otherwise, reperform the test, starting from the DNA isolation stage.

If blood volume exceeding 100 µl was used for DNA isolation (while maintaining calibration samples volume taken for DNA isolation), recalculate the concentration obtained: multiply the concentration value obtained by the ratio 100/V, where V is the used blood volume for DNA isolation.

Further principles of results interpretation are shown in Table 5. Concentration measurement accuracy: ± 0.5 lg concentration.

The reason for obtaining an invalid result may be the presence of inhibitors in the DNA obtained from clinical material, test protocol incorrect implementation, non-compliance with PCR temperature regime, etc.

The reason for obtaining a doubtful and negative result may be DNA insufficient concentration in the clinical sample.

If the result is invalid and doubtful, the conclusion is not issued. It is required to retake the biomaterial from the patient and retest it. However, for doubtful results, it is recommended to isolate DNA from a larger blood volume.

If a doubtful result is repeated, it is recommended to repeat the testing with a reagent kit from another manufacturer or by another method.

Table 5 – Result interpretation principle

Channels corresponding to fluorophores		Result interpretation
FAM / Green (*Plasmodium* spp.), IU/ml	HEX / Yellow (ICS), Ct	
$10^3 – 10^7$	not considered	The specific concentration in IU/ml in blood is indicated.
$< 10^3$	not considered	"less than 1000 IU/mL" is indicated
$> 10^7$	not considered	"more than 10^7 IU/mL" is indicated
absent	≤ 32	negative result (concentration is not indicated)
absent	absent	invalid result

Note: "absent" – there is no Ct value; "not considered" – Ct value is not taken into account.

23) Analytical efficiency characteristics

23.1 Analytical specificity

It is specific to 18S species of the genus *Plasmodium*, including *P. falciparum, P. vivax, P. malariae, P. ovale* (subspecies *curtisi* and *wallikeri*) and *P. knowlesi*.

There was no *in vitro* cross-reactivity: Chikungunya virus, Dengue 1 virus, Dengue 2 virus, Dengue 3 virus, Dengue 4 virus, Zika virus, human immunodeficiency virus 1 (HIV-1), human immunodeficiency virus 2 (HIV-2), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV); in *silico*: *Bartonella quintana*, *B. henselae*, *Borrelia bisetti*, *B. garinii*, *B. japonica*, *B. spielmanii*, *Coxiella burnetii*, *Dobrava-Belgrade orthohantavirus*, Japanese Encephalitis virus, *Leptospira interrogans*, *L. kirshneri*, *L. borgpetersenii*, Puumala orthohantavirus, *Rickettsia conorii*, *R. hejlonjiangensis*, Tick Borne Encephalitis Virus (TBEV), *Treponema pallidum*, *Trypanosoma cruzi*, West Nile virus, and Yellow Fever virus.

23.2 Analytical sensitivity

At least 500 IU/ml of whole blood DNA *Plasmodium* spp.

23.3 Precision under repeatability conditions

To evaluate precision under repeatability conditions, PC and ICS were tested using two fluorescence channels (FAM, HEX) in 10 repetitions each.

Repeatability data is 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 coefficient are calculated based on the values obtained in control samples repetitions.

According to the assay results the coefficient of variation under kit the repeatability conditions does not exceed 5%.

23.4 Precision under reproducibility conditions

Test system reproducibility is evaluated in a similar way to the precision calculation under repeatability conditions (Section 23.2). However, different batches of the reagent kit are used for testing, reactions are performed in different laboratories, by different operators, on different days, on different PCR cyclers (Reproducibility Unit 1, Reproducibility Unit 2, Reproducibility Unit 3, Reproducibility Unit 4).

There was complete intra-assay, inter-assay and inter-series reproducibility when conducting precision testing under reproducibility conditions, the coefficient of variation did not exceed 5%.

23.5 Limit of detection

At least 500 IU/ml of *Plasmodium* spp DNA. Linear range of detectable concentrations: $10^3 – 10^7$ IU/ml.

24) Clinical efficiency characteristics: diagnostic sensitivity and diagnostic specificity

Test material type	**Number of observations**	**Diagnostic sensitivity**	**Diagnostic specificity**	**Confidence interval** with 95% confidence probability
Blood		100%	100%	

25) Biological reference interval

Not applicable.

26) Information on interfering substances or limitations related to a sample that may affect the test result

The potentially interfering substances effect on *Plasmodium*-test-Q reagent kit performance has been tested in reference to potentially interfering substances that may be found during clinical material sampling in the following concentrations:

- hemoglobin – 10%;
- hyaluronic acid – 5%;
- Ibuprofen – 0.04 mg/ml;
- Ambrobene – 0.003 mg/ml;
- Bromhexine – 0.016 mg/ml;
- Kaletra – 0.02 mg/ml;
- Interferon – 0.2 U/mL;
- Teraflu – 0.071 mg/ml.

Based on the assay results, potentially interfering substances found during the DNA isolation from clinical material, evaluated at

concentrations that are expected to occur during *Plasmodium*-test-Q reagent kit normal use, do not affect the test result.

Limitations on test material use:

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

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

27) Warning and/or special precautions regarding the reagent kit and its accessories (if included) safe disposal

The reagent kit does not emit harmful substances polluting the environment during storage and transportation. Waste, originated from using a reagent kit for the intended purpose specified by the manufacturer, as well as unused kits (expired shelf life, damaged consumer packaging/labeling, damaged reagent packaging/labeling etc.) are classified as medical waste.

Medical waste must be collected, neutralized, placed, stored, transported, accounted and disposed in accordance with applicable rules and regulations.

Plasmodium-test-Q reagent kit consumer packaging is subject to mechanical destruction with the residues removal as industrial or household waste.

Personnel destroying a reagent kit must comply with the safety rules for a particular destruction method.

28) Warning and/or special precautions regarding a reagent kit designed for self-testing or near-patient testing

Not applicable.

29) Information on the last issue or the last version of the instructions for use

Version 1 dated 11.05.2024

30) Information about the need to contact a manufacturer or its authorized representative about undesirable events, having signs of an adverse event (incident).

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

Limited Liability Company TestGene (TestGene LLC),
9, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072, Russia
Phone number: +7 499 705 03 75
www.testgene.com
Technical Support Service:
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

Labelling symbols

Symbol	Symbol name
	Use before
	Manufacture date
	Batch code
	Refer to the instructions for use
	Temperature range
	Content is sufficient for 90 detections