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INSTRUCTIONS FOR USE

Reagent kit for human immunodeficiency viruses type 1 (HIV-1) RNA detection by RT-PCR-RT "HIV-1-test-Q"

TS 21.20.23-041-97638376-2021

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List of abbreviations

The following abbreviations and designations are used in these instructions:

PCR	polymerase chain reaction
RNA	ribonucleic acid
NC	negative control sample
PC	positive control sample
HIV	human immunodeficiency virus
ICS	internal control sample
CS-1	calibration sample No.1
CS-2	calibration sample No.2
SenC	sensitivity control
SC	specificity control

Introduction

HIV infection is an infectious anthroponotic chronic disease with contact transmission, caused by the human immunodeficiency virus, slowly progressing and characterized by damage to the immune system with the development of AIDS. Clinical manifestations of immune protection failure are opportunistic infections, malignant neoplasms, dystrophic and autoimmune processes, which, in the absence of specific treatment, lead to the death of the infected person[1].

Target analyte: HIV-1 genomic RNA specific region – the *pol* gene fragment.

The scientific validity of the target analyte lies in its specificity (RNA sequence uniqueness) in relation to the HIV-1 genome.

Human immunodeficiency virus (HIV) belongs to the Retroviridae family, the genus Lentiviruses. The genome is represented by a RNA molecule and includes about 9 000 nucleotides. HIV RNA detection indicates viral replication in the body and is a disease diagnostic method. The genetic material of the virus can be detected by RT-PCR 1-3 weeks after infection. Viral load quantitative detection is used to choose drugs as part of antiretroviral therapy regimens, to evaluate the antiretroviral therapy efficiency and for prognostic purposes. [1]

The scope of the reagent kit: clinical laboratory diagnostics of infectious diseases.

Indications and contraindications for use:

Indications for use: to diagnose HIV infection, to choose an antiretroviral therapy regimen, to monitor HIV infection progression and/or antiretroviral therapy efficiency, and it is also recommended for dispensary registration [1].

[1] Clinical recommendations "HIV infection in adults", year of approval: 2020. Ministry of Health of the Russian Federation.

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

Population, demographic aspects of the kit use: no population, demographic aspects of HIV-1-test-Q reagent kit use were identified.

Sterility: the kit is not sterile.

1. Intended use

Intended use: HIV-1-test-Q reagent kit is designed for human immunodeficiency virus (HIV-1) RNA quantitative detection by one-step polymerase chain reaction with real-time reverse transcription hybridization-fluorescence detection (RT-PCR-RT) in a RNA sample isolated from human blood plasma (containing EDTA-K2 as an anticoagulant), in patients to diagnose HIV to choose an antiretroviral therapy regimen, to monitor HIV infection progression and/or the antiretroviral therapy efficiency, and is also recommended for dispensary registration.

Functional purpose: the obtained results can be used to diagnose HIV infection, select an antiretroviral therapy regimen, monitor HIV infection progression and/or the antiretroviral therapy efficiency, and also for dispensary registration.

Potential consumers of a medical device:

The kit is intended for professional use in medical centers and clinical diagnostic laboratories. Professional level of potential users – clinical laboratory diagnostics doctor, medical technologist, medical laboratory technician.

2. Method principle

Method

One-step polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection with reverse transcription (RT-PCR-RT).

Test sample type

The material for the assay is RNA samples isolated from human blood plasma (containing EDTA-K2 as an anticoagulant).

Detection principle

Human immunodeficiency virus RNA quantitative detection by multiplex RT-PCR with hybridization-fluorescence detection in a RNA sample isolated from clinical material includes three stages:

1. RT-PCR preparation;
2. RNA reverse transcription and DNA PCR amplification with hybridization-fluorescent detection of amplification products in real-time;
3. Result interpretation.

One-step reverse transcription and amplification reactions of specific regions are carried out with RNA samples using primers specific to them in the reaction buffer.

RT-PCR Buffer contains all the basic reagents, including a warm-start revertase, a thermostable hot-start DNA polymerase, deoxynucleotide triphosphates and an optimized buffer.

The oligonucleotides mixtures contain fluorescently labeled oligonucleotide probes that hybridize with a complementary DNA region of the amplified target RNA and are hydrolyzed (destroyed) by *Taq* polymerase, as a result the fluorescence dye and quencher are separated and the fluorescence intensity an increases over the corresponding range of the optical spectrum. This allows to register the amplification specific product accumulation by measuring the fluorescent signal intensity in real time.

The kit contains reagents for highly specific regions (targets) of HIV-1 genomic RNA, as well as an internal control sample (ICS) detection (Table 1).

ICS allows to evaluate the RNA isolation quality and effectiveness and possible presence of amplification inhibitors in the sample, the presence of which can lead to false negative results.

Calibration samples CS-1 and CS-2 are used to draw the calibration curve required to determine HIV-1 genomic RNA concentrations in the test sample.

Table 1 – Test targets

Channel corresponding to the fluorophore	
FAM / Green	**HEX / Yellow**
HIV-1 RNA	ICS

Method limitations

A possible reason for obtaining a false positive result is contamination at RNA isolation or RT-PCR reaction stages. A false positive result can be detected with a negative control sample.

A reagent kit with an expired shelf life cannot be used.

Do not use the reagent kit if the inner packaging is damaged, or the reagent appearance does not match the description.

A reagent kit transported or stored in the temperature regime violation cannot be used.

The clinical diagnosis conclusion cannot be based on the assay results with this kit only. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical picture, the assay results from other test systems, the therapy used.

RT-PCR time ranges from 85 to 120 minutes (excluding sample preparation), depending on the cycler used.

3. Reagent kit components

HIV-1-test-Q reagent kit is designed in one configuration form – HIV-1-test-Q.

Test samples number

HIV-1-test-Q reagent kit is designed for 100 reactions, it equates to the detection of 88 test samples, calibration samples, negative and positive control samples with a single run of the 96-well cycler or 11 single test samples detections with calibration, negative and positive control samples in each test.

Reagent kit components

Table 2 – HIV-1-test-Q reagent kit components

No.	Reagent name	Description	Quantity, volume
1.	RT-PCR Buffer	Transparent colorless liquid	1 tube, 500 µl
2.	Oligonucleotide Mixture	Transparent, colorless liquid, may have a shade of lilac	1 tube, 500 µl
3.	PC	Transparent colorless liquid	1 tube, 165 µl
4.	NC	Transparent colorless liquid	1 tube, 1 100 µl
5.	ICS	Transparent colorless liquid	1 tube, 1 000 µl
6.	CS-1	Transparent colorless liquid	2 tubes 2 000 µl each
7	CS-2	Transparent colorless liquid	2 tubes 2 000 µl each

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 place a reagent kit in a reusable polyurethane foam thermal container with prepared ice packs for temporary storage and transportation. Place the thermal container, instructions for use and the quality certificate for each batch of products supplied into an individual packaging.

RT-PCR Buffer is ready for use and it is a reagent containing all the basic components, including a warm start revertase, thermostable hot start DNA polymerase, dNTP, an optimized buffer.

Oligonucleotide Mixture is ready for use and contains primers and probes designed to identify specific targets. Oligonucleotide Mixture is in a 10% aqueous solution of TE (1 mM Tris, 0.1 mM EDTA), nuclease-free.

PC (positive control sample) is ready for use and contains HIV-1 genome specific fragment and a bacteriophage genome fragment detected by a reagent kit at $1x10^{7}$ copies/ml concentration each. PC is in 10% TE buffer (10 mM Tris, 1 mM EDTA).

NC (**negative control sample)** is ready for use and is deionized DNases and RNases free water.

Calibration sample CS-1 is ready for use and contains HIV-1 genome specific fragment detected by a reagent kit with 1×10^{6} copies/ml concentration in a TE buffer (10 mM Tris, 1 mM EDTA).

Calibration sample CS-2 is ready for use and contains HIV-1 genome specific fragment detected by a reagent kit with $3x10^{3}$ copies/ml concentration in a TE buffer (10 mM Tris, 1 mM EDTA).

ICS (internal control sample) is ready for use and consists of armored RNA with 1.5×10^{6} copies/ml concentration in a TE buffer (10 mM Tris, 1 mM EDTA).

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

4. Reagent kit characteristics
4.1. Technical and functional characteristics

Table 3 – HIV-1-test-Q reagent kit

Indicator name	Characteristics and standards	Clause in TS
1. Technical characteristics		
1. Appearance		
RT-PCR Buffer	Transparent colorless liquid	Section 7, clause 7.6
Oligonucleotide Mixture	Transparent, colorless liquid, may have a shade of lilac color	Section 7, clause 7.6
PC	Transparent colorless liquid	Section 7, clause 7.6
NC	Transparent colorless liquid	Section 7, clause 7.6
ICS	Transparent colorless liquid	Section 7, clause 7.6
CS-1	Transparent colorless liquid	Section 7, clause 7.6
CS-2	Transparent colorless liquid	Section 7, clause 7.6
1.2. Completeness	Clause 1.4 TS 21.20.23-041-97638376-2021	Section 7, clause 7.9
1.3. Labelling	Clause 4 TS 21.20.23-041-97638376-2021	Section 7, clause 7.9
1.4. Packaging	Clause 5 TS 21.20.23-041-97638376-2021	Section 7, clause 7.9
2. Functional characteristics		
2.1 Positive result with PC	Fluorescence signal growth registered in tubes with PC in the channels FAM Ct≤30, HEX Ct≤30.	Section 7, clause 7.7.2
2.2 Negative result with NC	In tubes with NC in the FAM channel Ct is not indicated (i.e., there is no fluorescence accumulation curve) or Ct>35, and in the HEX channel Ct≤34	Section 7, clause 7.7.2
2.3 Reaction in tubes with SC	In tubes with SC in the FAM channel Ct is not indicated (i.e., no fluorescence accumulation curve) or Ct>35, and the HEX channel Ct≤34	Section 7, clause 7.7.2
2.4 Reaction in tubes with SenC	In tubes with SenC in the FAM channel, in all repetitions (at least 4) Ct≤35 and with a standard deviation in	Section 7, clause 7.7.2

	SenC repetitions not more than 5%, and in the HEX channel Ct≤34	
2.5 "Linearity" test	The correlation ratio of CS-1, CS-2 and the standard sample (SS) is at least 0.98	Section 7, clause 7.7.2
2.6 Precision test: coefficient of variation (CV) under repeatability conditions	The coefficient of variation Ct for repetitions of each calibration sample CS-1 and CS-2 under repeatability conditions does not exceed 5%.	Section 7, clause 7.7.2
2.6 Accuracy of concentration detection test	The obtained value of HIV-1 RNA concentration should correspond to the concentration given in the passport of the sample, with a tolerance of ±0.5 lg concentration.	Section 7, clause 7.7.2

In case of the medical device malfunction, deviations in its functioning that may affect safety, or changes in the kit analytical characteristics, stop using the device immediately and inform the manufacturer (see Section 14 of the Instructions).

4.2 Analytical efficiency characteristics

4.2.1 Analytical specificity

It is specific to human immunodeficiency virus 1 RNA of groups M (subtypes A–L), O, N, P.

The absence of nonspecific positive amplification results in the presence of the following organisms and viruses in the genomic NA sample was shown: hepatitis A, B, C and D viruses, cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, herpes virus types 6 and 8, varicella zoster virus, parvovirus B19, tick-borne encephalitis virus, West Nile virus, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. agalactiae*.

4.2.2 Limit of detection

The detection limit (LOD) was verified using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194.

According to the assay results, the HIV-1 RNA detection limit in 1000 µl K2-EDTA blood plasma samples with 95% detection rate using a cycler:

- DTprime – 17.5 copies/ml (95% CI: 16.1 – 18.9 copies/ml), 30.6 IU/ml (95% CI: 29.2 – 32.0 IU/ml)
- CFX 96 – 17.3 copies/ml (95%CI: 15.8 – 18.7), 30.2 IU/ml (95% CI: 28.7 – 31.6 IU/ml)
- Rotor-Gene Q – 16.7 copies/ml (95%CI: 15.2 – 18.1), 29.3 IU/ml (95% CI: 27.8 – 30.7 IU/ml)
- Quant Studio 5 – 17.7 copies/ml (95% CI: 16.2 – 19.1), 31.0 IU/ml (95% CI: 29.5 – 32.4 IU/ml)

Detection limit for testing various **HIV-1 subtypes: group M (A, B, C, D, AE, F, G, AG-GH), group N and group O** was verified using the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224. The results obtained confirmed HIV-1-test-Q reagent kit ability to detect subtypes of group M (A, B, C, D, AE, F, G, AG-GH), group N and group O at concentrations of ~ 20 copies/ml (~35 IU/ml) in 1000 µl K2-EDTA blood plasma samples with an upper one-sided 95% confidence interval, exceeding the expected 95% detection rate.

4.2.3 The limit of quantification

The quantification limit was verified using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194.

Based on the assay results, the HIV-1 RNA limit of quantification (LOQ) was determined in 1000 µl K2-EDTA plasma samples with 95% detection rate using a cycler:

- DTprime – 48.5 copies/ml (95% CI: 42.1 – 54.9 copies/ml), 84.9 IU/ml (95% CI: 74.1 – 95.6 IU/ml)
- CFX 96 – 47.8 copies/ml (95% CI: 41.3 – 54.2), 83.6 IU/ml (95% CI: 72.8 – 94.3 IU/ml)
- Rotor-Gene Q – 51.8 copies/ml (95%CI: 45.3 – 58.2), 90.7 IU/ml (95%CI: 79.9 – 101.4 IU/ml)
- Quant Studio 5 – 51.1 copies/ml (95%CI: 44.6 – 57.5), 89.4 IU/ml (95% CI: 78.6 – 100.1 IU/ml)

The quantification limit verification for testing various HIV-1 subtypes**: group M (A, B, C, D, AE, F, G, AG-GH), group N and group O** was carried out using the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224. The results obtained confirmed the limit of quantification (LOQ) of HIV-1-test-Q reagent kit in relation to subtypes **A, B, C, D, AE, F, G, AG-GH, group N and group O** at a concentration of ~ 50 copies/ml (~87.5 IU/ml) in 1000 µl K2-EDTA blood plasma samples with an upper one-sided 95% confidence interval, exceeding the expected 95% detection rate.

4.2.4 Linear measuring range

The linear measuring range was verified using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194.

According to the assay results, it can be concluded that for 1000 µl human K2-EDTA plasma samples the assay results with HIV-1-test-Q reagent kit are linear in the range from 50 copies/ml to 10^7 copies/ml (from 88 IU/ml to $1.75*10^7$ IU/ml) and demonstrate a maximum deviation from the regression line does not exceed ± 0.21 log10.

Linear range verification when testing HIV-1 different subtypes (A, B, C, D, AE, F, G, AG-GH, Group N and Group O) was conducted using the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224. The obtained results confirmed HIV-1-test-Q reagent kit linear measurement range when testing subtypes **A, B, C, D, AE, F, G, AG-GH, group N and group O**, a linear range from 90 IU/ml to $1.75*10^7$ IU/ml, the maximum deviation from the regression line does not exceed to 0.28 log10.

4.2.5 Precision under repeatability conditions

To evaluate precision under repeatability conditions, a positive control sample, calibration samples No. 1 and No. 2 and a sensitivity control sample were tested 10 repetitions each.

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

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

The assay results showed that the coefficient of variation under the kit repeatability conditions was 3% or less.

4.2.6 Precision under reproducibility conditions

The test system reproducibility is evaluated in a similar way to the calculation of precision under repeatability conditions, however, different reagent kit batches were used for testing, reactions were 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).

The assay results showed that the coefficient of variation under the kit reproducibility conditions did not exceed 5%.

4.2.7. Metrological traceability of control samples – PC, CS-1, CS-2 included in HIV-1-test-Q reagent kit was carried using **the WHO 4th International Standard for HIV-1, NIBSC code: 16/194**. The attributed concentration of CS-1 was 10^6 copies/ml ($1.75*10^6$ IU/ml), CS-2 – $3*10^3$ copies/ml (5250 IU/ml), PC – 10^7 copies/ml ($1.75*10^7$ IU/ml).

Based on the results of the calibration and standardization process, it can be concluded that HIV-1-test-Q reagent kit provides quantitative values for the WHO 4th International Standard for HIV-1, NIBSC code: 16/194, which are similar to the expected values with a deviation of not exceeding ±0.71 log10 copies/ml (uncertainty).

4.3. Clinical efficiency characteristics

During the clinical trials, 141 human blood plasma samples (containing EDTA-K2 as an anticoagulant) were used from patients diagnosed with HIV infection aged 4 to 63 years.

The biological reference range of HIV-1 RNA concentration in the tested population was from 123 copies/ml to 97723 copies/ml (2.09 log10 copies/ml to 4.99 log10 copies/ml).

To evaluate cross-reactivity in clinical trials with the tested reagent kit HIV-1-test-Q **37 samples**, that did not contain HIV-1 RNA, but with confirmed genomic DNA positive presence of the following organisms and viruses, were also tested: hepatitis A, B, C and D viruses, cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, herpes virus types 6 and 8, varicella zoster virus, parvovirus B19, tick-borne encephalitis virus, West Nile virus, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. agalactiae*.

This number of samples was selected taking into account **GOST R 51352-2013** requirements and taking into account the International Guideline **CLSI EP09-A3** recommendations.

Each sample was tested in two series using the tested reagent kit for human immunodeficiency viruses type 1 (HIV-1) RNA detection by RT-PCR-RT "HIV-1-test-Q" according to TS 21.20.23-041-97638376-2021, produced by TestGene LLC and a comparison kit AmpliSens® HIV-Monitor-FRT, Russia (RU No. FSR 2008/02552 dated November 22, 2019).

To conduct a PCR assay using HIV-1-test-Q test kit, the cyclers recommended by the reagent kit manufacturer were used:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

RNA isolation from clinical samples was carried out by FSBI Federal Clinical Research Center for Specialized Medical Care and Medical Technologies of the Federal Medical and Biological Agency of Russia using RNA isolation kits recommended in the tested HIV-1-test-Q kit Instructions for Use:

- A reagent kit for RNA/DNA extraction from biological material "MAGNO-sorb" according to TS 9398-106-01897593-2012,

manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate no. FSR 2010/07265 dated March 21, 2022).

4.3.1 Method comparison: accuracy.

The data obtained during the testing **of 141 human blood plasma samples** (containing EDTA-K2 as an anticoagulant) from patients with confirmed HIV infection diagnosis allow to conclude on reliable compliance of the HIV-1 RNA concentration quantitative detection results in clinical samples obtained with **the tested reagent kit** for human immunodeficiency viruses type 1 (HIV-1) RNA detection by RT-PCR-RT "HIV-1-test-Q" according to TS 21.20.23-041-97638376-2021, produced by TestGene LLC and a **comparison kit** "AmpliSens® HIV-Monitor-FRT", manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate no. FSR 2008/02552 dated November 22, 2019) during PCR assay using **cyclers:**

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate no. FSR 2011/10228 dated March 03, 2011;
- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;
- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated August 10, 2010;
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019.

The systematic error in measuring the HIV-1 RNA concentration logarithm does not exceed 3%.

The statistical processing results of the obtained data on methods comparison (accuracy) according to CLSI EP09-A3 document recommendations using the regression and correlation method.

	Sample type	**Unit**	**Used cycler**	**Number of samples**	**Correlation ratio**	**Intersection**	**Slope**
HIV-1-test-Q reagent kit, produced by TestGene LLC **in comparison** with AmpliSens® HIV-Monitor-FRT, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate no. FSR 2008/02552 dated 22.11.2019)	Human plasma (with EDTA-K2 as an anticoagulant	log10 copies /ml	DTprime	141	0.9983	0.0056	0.998
			CFX 96	141	0.9981	0.0154	0.9961
			Rotor-Gene Q	141	0.9978	-0.0094	1.0028
			Quant Studio 5	141	0.998	-0.0048	1.0006

4.3.2 Inter-lot correlation detection results (human blood plasma K2-EDTA).

To **determine inter-lot correlation** of measurement results in clinical samples in accordance with the international guideline CLSI EP09-A3, a scattering diagram of the dependent variable X - HIV–1 RNA concentration was drawn using the tested "HIV-1-test-Q" reagent kit, manufactured by TestGene LLC, **LOT: 202207-273**, and Y - HIV RNA-1 concentration using the tested "HIV-1-test-Q" reagent kit, manufactured by TestGene LLC, **LOT: 202207-274**.

The statistical processing results of the obtained data on the inter-lot correlation detection in accordance with recommendations of the CLSI EP09-A3 document using the regression and correlation method.

	Sample type	Unit	Cycler used	Number of samples	Correlation ratio	Intersection	Slope
HIV-1-test-Q reagent kit, produced by TestGene LLC **LOT: 202207-273 in comparison** with **LOT: 202207-274**	Human blood plasma (with EDTA-K2 as an anticoagulant	log10 copies/ml	DTprime	141	0.9912	-0.0023	0.9987
			CFX 96	141	0.9914	0.0184	0.9948
			Rotor-Gene Q	141	0.9919	-0.0095	1.0051
			Quant Studio 5	141	0.9923	0.0303	0.9914

Correlation ratio R^2 during testing on each cycler used was more than **0.99.** In accordance with the CLSI EP09-A3 document recommendations, using the regression and correlation method, it can be concluded that correlation strength of HIV RNA concentration is high in clinical samples obtained using **two lots of the tested reagent kit** for human immunodeficiency viruses type 1 (HIV-1) RNA detection in by RT-PCR-RT "HIV-1-test-Q" according to TS 21.20.23-041-97638376-2021, produced by TestGene LLC.

4.3.3 Specificity evaluation

4.3.3.1 In vitro specificity evaluation using the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224

An international reference sample was used to evaluate detection and quantification possibility of equally different subtypes (A, B, C, D, AE, F, G, AG-GH, group N and Group O):

- International standard **2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code:**

12/224, consisting of 10 samples covering the most common HIV-1 subtypes: sample 1 (subtype A), sample 2 (subtype B), sample 3 (subtype C), sample 4 (subtype D), sample 5 (subtype AE), sample 6 (subtype F), sample 7 (subtype G), sample 8 (subtype AG-GH), sample 9 (group N), sample 10 (group O).

For the assay recombinant positive samples were prepared by diluting samples of the **NIBSC code: 12/224** panel in HIV-1 RNA negative human K2-EDTA plasma from individual donors. HIV-1 RNA absence status in clinical samples was established using:

- A reagent kit for simultaneous detection of hepatitis C virus (HCV) RNA, hepatitis B virus (HBV) DNA and human immunodeficiency virus (HIV) RNA in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® HCV/HBV/HIV-FL" according to TS 9398-069-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, RU No. FSR 2009/06187 dated February 26, 2019.

Each sample was tested in two duplicates in each testing cycle (a complete testing procedure for each repetition, including RNA isolation prior to amplification and detection).

The assay results confirmed the specificity evaluation of HIV-1-test-Q reagent kit equally to various subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O) HIV-1.

4.3.3.2 Product specificity: cross-reactivity evaluation

To evaluate cross-reactivity in clinical trials with the tested reagent kit HIV-1-test-Q **37 samples,** that did not contain HIV-1 RNA, but with the confirmed genomic DNA positive presence of the following organisms and viruses, were also tested: hepatitis A, B, C and D viruses, cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, herpes virus types 6 and 8, varicella zoster virus, parvovirus B19, tick-borne encephalitis virus, West Nile virus, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. agalactiae.*

In accordance with the tested HIV-1-test-Q reagent kit Instructions for Use, RT-PCR-RT series was performed and the reaction with the tested clinical samples not containing HIV-1 RNA was evaluated.

To carry out a PCR assay using the tested HIV-1-test-Q kit, the cyclers recommended by the reagent kit manufacturer were used:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

Negative results were obtained for all clinical samples tested, confirming the **absence of non-specific positive results** in relation to NA of the following organisms and viruses: hepatitis A, B, C and D viruses, cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, herpes virus types 6 and 8, varicella zoster virus, parvovirus B19, tick-borne encephalitis virus, West Nile virus, *Escherichia coli*, *Staphylococcus aureus, Streptococcus pyogenes, S. agalactiae*.

4.3.4 HIV-1-test-Q reagent kit detection limit evaluation

4.3.4.1 Confirm the detection limit using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194

According to GOST R 51352-2013 and taking into account the **CLSI EP-17A2** international recommendations, the detection limit (LOD) was determined by dilution testing of the WHO **4th International Standard for HIV-1, NIBSC code: 16/194** in the range of the estimated detection limit – 3, 5, 7, 10, 15, 17, 20 copies/ml (5,2, 8,7, 12,2, 17,5, 26,2, 29.7, 35 IU/ml).

To evaluate the detection limit, **the WHO 4th International Standard for HIV-1, NIBSC code: 16/194** was diluted in HIV-1 RNA negative human K2-EDTA plasma from individual donors.

Each sample in 7 dilutions was tested using HIV-1-test-Q kit for 3 different days in 30 repetitions to calculate the positive results percentage. The results were determined in accordance with the **CLSI EP-17A2** international recommendations by probit analysis.

Based on the assay results the manufacturer's stated detection limit of HIV-1 RNA in 1000 µl K2-EDTA plasma samples with 95% detection rate was confirmed for the cycler:

- DTprime – 16.6 copies/ml (95%CI: 15.1 – 18.0 copies/ml), 29.0 IU/ml (95% CI: 27.5 – 30.4 IU/ml)
- CFX 96 – 17.9 copies/ml (95% CI: 16.4 – 19.3), 31.1 IU/ml (95%CI: 29.6 – 32.5 IU/ml)
- Rotor-Gene Q – 16.9 copies/ml (95%CI: 15.4 – 18.3), 29.5 IU/ml (95% CI: 27.1 – 30.9 IU/ml)
- Quant Studio 5 – 18.1 copies/ml (95% CI: 16.6 – 19.5), 31.7 IU/ml (95%CI: 30.27 – 33.1 IU/ml).

4.3.4.2 The detection limit verification when testing various HIV-1 subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O)

To verify the detection limit when testing various HIV-1 subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O), samples from **the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224** containing HIV-1 RNA samples of ten different subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O) were used.

To verify the detection limit when testing various HIV-1 subtypes, samples from **the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224** were diluted in HIV-1 RNA negative 1000 µl human K2-EDTA plasma from individual donors to the LoD concentration set using the WHO 4th International Standard for HIV-1 NIBSC code: 16/194 (HIV-1 subtype B) ~ 20 copies/ml (~35 IU/ml).

The following cyclers were used for the PCR assay:

- DTprime detecting cycler (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

The obtained results confirmed HIV-1-test-Q reagent kit ability to detect HIV-1 subtypes: A, B, C, D, AE, F, G, AG-GH, group N and group O at ~ 20 copies/ml (~35 IU/ml) concentrations in 1000 µl K2-EDTA plasma samples with an upper one-sided 95% confidence interval, exceeding the expected 95% detection rate.

4.3.5 HIV-1-test-Q reagent kit limit of quantification evaluation

4.3.5.1 Confirm the limit of quantification using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194

According to GOST R 51352-2013 and taking into account **CLSI EP-17A2** international recommendations, on quantitative detection (LOQ) LoQ was determined by the WHO 4th **International Standard for HIV-1, NIBSC code: 16/194** dilution testing method within the range of the estimated detection limit – 10, 20, 30, 35, 40, 45, 50 copies/ml (17.5; 35; 52.5; 61.2; 70; 78.7; 87.5 IU/ml).

To evaluate the detection limit, **the 4th WHO International Standard for HIV-1, NIBSC code: 16/194** was diluted in HIV-1 RNA negative human K2-EDTA plasma from individual donors.

Based on the assay results, the manufacturer's stated limit of quantification (LOQ) of HIV-1 RNA in 1000 µl K2-EDTA blood plasma samples with 95% detection rate was confirmed for the cycler:

- DTprime – 48.1 copies/ml (95% CI: 41.6 – 54.5 copies/ml), 84.2 IU/ml (95%CI: 73.4 – 94.9 IU/ml)
- CFX 96 – 48.9 copies/ml (95% CI: 42.4 – 55.3), 85.6 IU/ml (95%CI: 74.8 – 96.3 IU/ml)
- Rotor-Gene Q – 48.3 copies/ml (95%CI: 41.8 – 54.7), 84.6 IU/ml (95%CI: 73.8 – 95.3 IU/ml)
- Quant Studio 5 – 49.8 copies/ml (95%CI: 43.3 – 56.2), 87.2 IU/ml (95% CI: 76.4 – 97.9 IU/ml).

4.3.5.2 The detection limit verification when testing various HIV-1 subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O)

To verify the detection limit when testing various HIV-1 subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O), samples from **the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224** containing HIV-1 RNA

samples of ten different subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O) were used.

To verify the detection limit when testing various HIV-1 subtypes, samples from **the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224** were diluted in HIV-1 negative 1000 µl human K2-EDTA plasma from individual donors to a concentration of LoD set with the WHO 4th International Standard for HIV-1, NIBSC code: 16/194 (HIV-1 subtype B) ~ 20 copies/ml (~35 IU/ml).

The obtained results confirmed HIV-1-test-Q reagent kit ability to detect HIV-1 subtypes: A, B, C, D, AE, F, G, AG-GH, group N and group O at ~ 20 copies/ml (~35 IU/ml) concentration in 1000 µl K2-EDTA blood plasma samples with an upper one-sided 95% confidence interval, exceeding the expected 95% detection rate.

4.3.6 Linear measuring range check

4.3.6.1 Linear measuring range check using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194

The HIV-1-test-Q linearity test according to GOST R 51352-2013 was determined by testing a series of dilutions, which included 12 panel samples covering the expected linear range. High-titer panel samples were prepared from a high-titer recombinant positive sample, panel samples with a lower titer were prepared by dilution of **the WHO 4th International Standard for HIV-1, NIBSC code: 16/194**, with 125900 IU/ml (220325 copies/ml) concentration.

The linear panel was prepared to provide covering of approximately 0.5 log10 copies/ml between the samples: 10^7 copies/ml, $10^{6.5}$ copies/ml, 10^6 copies/ml, $10^{5.5}$ copies/ml, 10^5 copies/ml, $10^{4.5}$ copies/ml, 10^4 copies/ml, $10^{3.5}$ copies/ml, 10^3 copies/ml, $10^{2.5}$ copies/ml, 10^2 copies/ml, 50 copies/ml.

For evaluation, the prepared control samples of the linear panel were diluted in HIV-1 RNA-negative human K2-EDTA plasma from individual donors.

Based on the results of the linear range assay, it can be concluded that for 1000 µl human K2-EDTA plasma samples, the assay results with HIV-1-test-Q reagent kit are linear in the range from 50 copies/ml to 10^7 copies/ml (from 88 IU/ml to $1.75*10^7$ IU/ml) and demonstrate a maximum deviation from the regression line not exceeding ± 0.21 log10.

4.3.6.2 Linear measuring range check when testing different HIV-1 subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O)

To verify the linear measurement range when testing HIV-1 various subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O), samples from **the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) NIBSC code: 12/224** containing HIV-1 RNA samples of ten different subtypes (A, B, C, D, AE, F, G, AG-GH, group N and group O) were used.

Based on the results of a linear range assay when testing HIV-1 various subtypes **(A, B, C, D, AE, F, G, AG-GH, group N and group O)**, it can be concluded that for 1000 µl human K2-EDTA plasma samples the test results with HIV-1-test-Q reagent kit are linear in the range from 50 copies/ml to 10^7 copies/ml (from 88 IU/ml to $1.75*10^7$ IU/ml) and demonstrate a maximum deviation from the regression line not exceeding ± 0.21 log10.

4.3.7 Kit precision evaluation: under repeatability and reproducibility conditions

4.3.7.1 Precision determination under repeatability conditions

The precision under repeatability conditions of the test system was evaluated by sequential assays of 3 positive clinical samples (low titer, mid-range titer and high titer) and 1 negative sample.

Based on the results of the precision under repeatability conditions assay, it can be concluded that the HIV-1-test-Q reagent kit coefficient of variation under repeatability conditions does not exceed 3%.

4.3.7.2 Precision determination under reproducibility conditions

The precision under reproducibility conditions of the test system was evaluated by sequential assays of 3 positive clinical samples (low titer, mid-range titer and high titer) and 1 negative sample.

Based on the results of a precision under reproducibility conditions assay, it can be concluded that the HIV-1-test-Q reagent kit coefficient of variation under reproducibility conditions does not exceed 5%.

4.3.8 Interfering substances effect evaluation

Based on the assay results, the following substances were classified as PCR inhibitors during the assay:

1) anticoagulants – heparin at 0.15 IU/ml concentration and sodium citrate at 0.1 mM/ml concentration. It is not allowed to use heparin and sodium citrate as an anticoagulant when taking peripheral blood.

2) heparin at 1 IU / ml concentration, used in anticoagulant therapy. Heparin presence in patients' blood undergoing anticoagulant therapy may lead to inaccurate PCR results, therefore, it is recommended to collect blood from such patients before the next administration of the drug.

Other interfering substances at the specified interferent concentrations do not affect the test results. A negative result in the HIV-1-test-Q test was obtained for all HIV-1 RNA-negative samples, and a positive result was obtained for all HIV-1 RNA-positive samples. In addition, the average log10 titer of each HIV-1-positive sample containing potentially interfering substances was between -0.19 log10 and 0.22 log10 of the average log10 titer of the corresponding positive sample.

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. Loss of reagents functional properties due to transportation, storage or use under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding permissible levels;
3. Reaction mixtures and tested RNA samples contamination with contents from a PC tube or amplification products;
4. Testing with a poor-quality RNA sample (low concentration and/or poor purification);
5. Failure to comply with the requirements for sample preparation, testing and disposal due to the unqualified personnel work;
6. Use of an unsuitable kit (use after the expiration date or in case of packaging damage).

No risks were identified in the unacceptable risk zone.

The cumulative residual risk of using HIV-1-test-Q reagent kit is acceptable, and the benefits of its use exceed the risk.

6. Safety precautions

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

All components and reagents included in HIV-1-test-Q reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards requirements. Harmful substances. Classification and general safety requirements".

Reagents included in HIV-1-test-Q kit have low vapor elasticity and exclude the possibility of inhalation poisoning.

Reagents included in HIV-1-test-Q kit are non-toxic, as they are

prepared by mixing individual non-toxic components.

Work with material infected or suspected of infection is carried out in accordance with the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases", MU "Work organization of laboratories using methods of nucleic acid amplification when working with material containing microorganisms of pathogenicity groups I–IV" (MU 1.3.2569-09).

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 tested samples.

The work should be carried out in a laboratory performing molecular biological (PCR) assays of clinical material in compliance with sanitary and epidemiological rules of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow methodological recommendations "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" (MU 287-113), MU "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I–IV" (MU 1.3.2569-09).

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 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 implementation of sanitary and anti-epidemic (preventive) measures";

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and slash 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;

– allow only specially trained personnel to work with the kit (a

specialist with higher medical education who has been trained in licensed specialization courses for working with pathogenic biological agents of pathogenicity groups I–II and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

– do not use the kit after the expiration date;

– do not use the reagent kit if the inner packaging is damaged, or the reagent appearance does not match the description;

– avoid contact with skin, eyes and mucosa; in case of contact, rinse immediately 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, sources of thermal inflammation are not provided.

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

7. Required equipment and materials

Work with a reagent kit is carried out in the working area 3 (for reactions preparation) (MU 1.3.2569-09).

Multiplex PCR equipment:

1. Class II and III biological safety cabinet;
2. Vortex;
3. Variable volume dispensers allowing to take liquid volumes of 20–200 µl, 200–1000 µl;
4. Refrigerator from 2°C to 8°C with freezer below than -16°C;
5. Cycler[2] with real-time fluorescence detection in channels corresponding to FAM/Green and HEX/Yellow fluorophores: CFX96 (BioRad, USA), DTprime, (NPO DNA Technology LLC, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

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

Materials and reagents not included in the kit:

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic RNase-free consumables.

1. Disposable pipette tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (for example, Axygen, USA);
2. Disposable sterile Eppendorf type 1.5 or 2.0 ml tubes;
3. Thin-walled disposable PCR tubes with optically transparent lid (when using plate type cyclers) or optically transparent walls (when using rotary type cyclers): 0.1 or 0.2 ml PCR tubes, or 0.1 or 0.2 ml PCR tubes strips, or PCR plates with an optically transparent film (for example, Axygen, USA) compatible with the used cycler;
4. Lab coat and talc-free disposable 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;
7. Kit for RNA isolation from blood plasma (see Section 8.2 of the Instructions);
8. Saline solution, TE buffer or deionized water (for diluting calibrators when isolating from a volume exceeding 100 µl).

8.Test samples

Test sample type

The material for the assay is RNA samples isolated from human blood plasma (containing EDTA-K2 as an anticoagulant).

8.1. Clinical material sampling

ATTENTION! Before starting work, study the methodological recommendations "Taking, transporting and storing clinical material for PCR diagnostics" developed by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Material sampling for assay

4 or 6 ml peripheral blood is taken in the morning on an empty stomach into a test tube (vacuum tube) containing EDTA-K2 solution as an anticoagulant. Turn the tube upside down 3-4 times to mix the blood with the EDTA-K2 solution right after blood sampling

ATTENTION! Heparin and sodium citrate use as an anticoagulant is not allowed.

ATTENTION! Heparin presence in blood of the patients undergoing anticoagulant therapy can lead to inaccurate RT-PCR results,

therefore, it is recommended to collect blood from such patients before the next administration of the drug.

Transportation and storage conditions of initial clinical material – blood:

– at +2°C... +8°C – up to 6 hours;

– at room temperature – up to 2 hours.

Do not freeze the blood.

Plasma should be isolated within 2 hours (when stored at room temperature) or 6 hours (when stored at from +2°C... +8°C) after material sampling. For that centrifuge the tube with blood at 800-1600 g for 20 minutes at room temperature. After centrifugation, transfer the upper fraction (plasma) into a separate 1.5 or 2.0 ml plastic RNase-free tube.

Blood plasma transportation and storage conditions:

Plasma can be stored at +2°C... +8°C up to 5 days, at -18°C... -22°C up to 3 months, at -70°C for a long time.

ATTENTION! Avoid repeated freezing and thawing of plasma samples.

To isolate RNA, use at least 100 µl of plasma.

An increase of the kit analytical sensitivity is possible due to the use a larger plasma volume (1000 µl) if this is provided by the used RNA isolation kit, aa well as a decrease of the elution volume.

Material pre-processing

No preparation is required.

Accounting, storage, transfer and transportation of clinical material suspected of HIV-1 presence should be carried out in accordance with the current sanitary rules and regulations of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases". Clinical material (Class B) disposal, as extremely epidemiologically hazardous waste, is carried out in accordance with SanPiN 2.1.3684-21.

8.2. Human RNA sample isolated from blood plasma collection

To isolate a RNA sample from human blood plasma, it is recommended to use the following reagent kits:

A reagent kit for RNA/DNA isolation from biological material "MAGNO-sorb" according to TS 9398-106-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate no. FSR 2010/07265 dated March 21, 2022).

During the RNA isolation procedure, the protocol and the instructions of the reagent kit used must be strictly followed.

Add 10 µl of ICS from HIV-1-test-Q reagent kit to the plasma intended for RNA isolation,

100 µl of NC (if necessary, increase the NC volume to the required using sterile saline solution) and calibration samples CS-1 and CS-2 in a volume corresponding to the volume of the tested clinical material also undergo the isolation stage with the addition of 10 µl of ICS. Meanwhile, it is required to add appropriate volume of saline solution, or TE buffer, or deionized water to 100 µl of each calibration sample. It is required to recalculate CS-1 and CS-2 concentration using the formulas:

for CS-1: $C = 10^6 * 100 / V$, where V is required calibrator volume in µl;

for CS-2: $C = 3*10^3 * 100 / V$, where V is required calibrator volume in µl.

Tested RNA samples storage conditions:

- at +2 ...+8°C up to 4 hours (recommended),
- at -18... -22°C up to one week,
- at a temperature below -80°C up to one year.

8.3. Interfering substances and limitations on the test material use

The effect of potentially interfering substances on HIV-1-test-Q reagent kit performance was studied regarding the potentially interfering substances that may occur during HIV-1-test-Q reagent kit normal use and presumably affect the reagent kit ability to produce valid results.

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

1) substances used in a patient's treatment (for example, medicines);

2) substances found in specific sample types – in this case, clinical sample contamination with blood hemoglobin can inhibit PCR if not sufficiently purified during the RNA isolation procedure;

3) substances found during the clinical material sampling procedure - in this case, anticoagulants.

The studied concentrations of interfering substances are shown in Table 6.

Table 6 – Interfering substances

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 µg/ml
Triglycerides	37 mmol/l
Bilirubin	210 µmol/l
Heparin (anticoagulant)	0.15 IU/ml
Sodium Citrate (anticoagulant)	0.1 mM/ml
EDTA-K2 (anticoagulant)	0.5 mM/ml
Exogenous interfering substances	
Anticoagulant therapy	
Heparin	1 IU/ml
Medications prescribed for HIV-1 therapy	
Efavirenz	0.12 mg/ml
Dolutegravir	0.02 mg/ml
Lamivudine	0.06 mg/ml

Tenofovir	0.06 mg/ml
Emtricitabine	0.04 mg/ml
Elsulfavirine	0.004 mg/ml
Phosphazide	0.16 mg/ml
Lopinavir	0.16 mg/ml
Abacavir	0.12 mg/ml
Dolutegravir	0.01 mg/ml
Zidovudine	0.12 mg/ml
Raltegravir	0.16 mg/ml
Azithromycin	0.1 mg/ml

Based on the study results, the following substances were classified as PCR inhibitors during the assay:

1) anticoagulants – heparin at 0.15 IU/ml concentration and sodium citrate at 0.1 mM/ml concentration. It is not allowed to use heparin and sodium citrate as an anticoagulant when taking peripheral blood.

2) heparin at 1 IU / ml concentration, used in anticoagulant therapy. Heparin presence in patients' blood undergoing anticoagulant therapy can lead to inaccurate PCR results, therefore, it is recommended to collect blood from such patients before the next administration of the drug.

To reduce the PCR inhibitor amount, it is required to follow the instructions for clinical material sampling.

Limitations on test material use:

– the test material cannot be used in case of storage and transportation conditions violation (temperature, duration, multiple freezing and thawing);

– blood plasma samples collected in test tubes with heparin or sodium citrate as an anticoagulant are not suitable for testing;

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

– heparin presence in patients' blood undergoing anticoagulant therapy may lead to inaccurate PCR results, therefore, it is recommended to collect blood from such patients before the next administration of the drug.

9. Kit components preparation for testing

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

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic RNase-free consumables. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

ATTENTION! Mix the reaction mixture components right before the testing.

Before preparing the reaction mixtures, it is necessary to wet clean the PCR box, as well as the equipment and materials contained in it, with disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes. Before the test, it is necessary to defrost the kit components at room temperature.

1. Mix thoroughly the tubes contents with the RNA isolated for testing, RT-PCR Buffer, Oligonucleotide Mixture, CS-1, CS-2, NC and PC, turning upside down each tube 10 times or mixing on a vortex at low speed for 3-5 seconds, then remove drops from the tube lids by short centrifugation.

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

10. Testing procedure

The PCR test consists of the following stages:

1. RT-PCR preparation;
2. RNA reverse transcription and DNA PCR amplification with hybridization-fluorescent detection of amplification products in real-time;
3. Result interpretation.

A) RT-PCR preparation

(carried out in pre-PCR area – a room for reagent dispensing and preparation for PCR amplification)

Total reaction volume – 25 µl.

3 To improve accuracy, it is recommended to analyze each sample twice.

ATTENTION! It is forbidden to change the reaction volume.

To prepare a reaction mixture for 1 reaction, you need:

1. RT-PCR Buffer – 5 µl,
2. Oligonucleotide mixture – 5 µl,
3. Sample (test RNA, PC, NC) – 15 µl.

Prepare reaction tubes as follows:

1. Label 0.1 or 0.2 ml PCR tubes.
2. In a separate 1.5 or 2.0 ml disposable sterile Eppendorf type tube prepare a reaction mixture: (n+9) x5 µl of RT-PCR Buffer and (n+9) x5 µl of Oligonucleotide Mixture, where n is the number of test samples.
3. Add 10 µl of the prepared reaction mixture into each PCR tube.
4. Add 15 µl of isolated RNA into the corresponding tubes for the test samples. Do not add RNA into the PC and NC tubes.
5. Add 15 µl of CS-1, which has passed the isolation stage, into 3 corresponding tubes.
6. Add 15 µl of CS-2, which has passed the isolation stage, into 3 corresponding tubes.
7. Add 15 µl of PC into the corresponding tube.
8. Add 15 µl of NC, which has passed RNA isolation stage, into the corresponding (see Section 8.2).
9. To remove drops from the walls, centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge.

B) RNA reverse transcription and DNA PCR amplification with hybridization-fluorescence detection of amplification products in real time;

(carried out in PCR area – a room for PCR amplification)

1. Place the tubes in the reaction module of the real-time PCR device. It is recommended to place the tubes in the thermoblock center to press evenly the tubes with a heating lid.
2. Program the device to perform the appropriate PCR program and detect the fluorescent signal, following the instructions for the device used. Test type: quantitative with standards. PCR protocol is shown in Table 7. If simultaneous testing with reagent kits HEPA-BCD-test (No. RZN 2022/16782 dated March 29, 2022), HEPA-B-test-Q (No. RZN 2022/16949 dated April 18, 2022), HEPA-C-test-Q (No. RZN 2022/16797 dated April 1, 2022) is required, use the protocol in Table 8.

ATTENTION! If DTprime and similar cyclers are used, the exposure value in the FAM channel should not exceed 1000.

Table 7 – RT-PCR protocol

Stage	Temperature, °C	Time, min.:sec.	Detection channels	Total number of cycles
1	52	25:00	–	–
2	95	02:00	–	–
3	95	00:05	–	5
	60	00:15	–	
	67	00:15	–	
4	95	00:05	–	42
	60	00:15	FAM/Green, HEX/Yellow	
	67	00:15	–	

Table 8 – RT-PCR protocol when performed simultaneously with HEPA-BCD-test, HEPA-B-test-Q, HEPA-C-test-Q reagent kits

Stage	Temperature, °C	Time, min.:sec.	Detection channels	Total number of cycles
1	52	40:00	–	–
2	95	02:00	–	–
3	95	00:05	–	5
	60	00:15	–	
	67	00:30	–	
4	95	00:05	–	45
	60	00:15	FAM/Green, HEX/Yellow	
	67	00:30	–	

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

4. Make sure that the FAM/Green and HEX/Yellow detection channels are included the optical measurement parameters of the amplification program.

5. Start PCR with fluorescent signal detection.

6. Upon the program completion, analyze the results.

11. Result registration and interpretation

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

Recommendations on setting the threshold line

For cyclers of any models, 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 Ct values of the FAM/Green and HEX/Yellow channels. Only Ct values obtained at the PCR stage with fluorescence detection are taken into account (that is, corresponding to stage 4 – see Table 7).

First, the reaction outcome and Ct values in the control samples are evaluated. Result interpretation in test samples begins only after the correct PC and NC outcome.

ATTENTION! In case of using Rotor-Gene Q cycler, activate functions “Dynamic Tube”, “Noise slope correction”, set 10% value in the “Outlier Removal” section.

Result interpretation in control samples

The following results should be obtained for NC and PC (Table 9).

Table 9 – Assay results for NC and PC

Control sample	Ct values for detection channels corresponding to fluorophores	
	FAM/Green	HEX/Yellow
NC	> 35 or absent	≤ 34
PC	≤ 30	≤ 30

When obtaining values for NC that differ from those indicated in Table 9, the results of the entire series are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

When obtaining values for PC that differ from those indicated in Table 9, repeat amplification of the entire sample batch. When reobtaining values for PC that differ from those indicated in Table 9, it is required to replace the reagents.

Result interpretation.

The result 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, it is necessary to draw a calibration curve. The test samples concentrations are calculated using the calibration curve.

Ct values ≤ 35 in the FAM channel are taken into account for the samples. If Ct > 35 for the samples (when the Ct value of ICS ≤ 34), the result is considered doubtful.

PCR efficiency should be in 85 to 115% range, and the difference between the Ct values of the repetitions of each calibration sample, CS-1 and CS-2, should be no more than 1.5. Otherwise, it is necessary to repeat the test, starting from the RNA isolation stage. If one of three CS-1 or CS-2 duplicates has a Ct that value deviates sharply from the others, it is allowed to ignore it when drawing the calibration curve.

Further result interpretation principles are shown in Table 10.

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

The reason for obtaining a doubtful result may be an insufficient virus concentration in the clinical sample.

Table 10 – Result interpretation principle

Channels corresponding to fluorophores		Result interpretation.
FAM/Green (HIV-1), copies/ml	HEX/Yellow (ICS), Ct	
50 – 10^{7*}	not considered	A positive result with indication of a specific concentration in copies/ml
< 50*	not considered	A positive result with indication "less than 50 copies/ml"
> 10^{7*}	not considered	A positive result with indication "more than 10^{7} copies/ml"
–	≤ 34	negative result (concentration is not indicated)
–	–	invalid result

Note: "not considered" – the result is not taken into account during interpretation; "–" – there is no fluorescence signal, the concentration is not specified; * values are given when isolated from 1000 µl of plasma

In case of an invalid and doubtful result, a conclusion is not issued, it is necessary to retake the biomaterial from the patient and retest it. However, for doubtful results, it is recommended to isolate RNA from a larger plasma volume.

If a doubtful result repeats, repeat the test with a reagent kit from another manufacturer or by another method.

If it is necessary to present the result in international units (IU), it is recommended to use the coefficient: 1 copy/ml = 1.75 IU/ml, defined using the WHO 4th International Standard for HIV-1, NIBSC code: 16/194.

The diagnostic value of the obtained test result:

The test result can be used by a qualified specialist (doctor) taking into account the clinical picture and other test types data in combination, to diagnose HIV infection, choose an antiretroviral therapy regimen, monitor the HIV infection progression and/or the antiretroviral therapy effectiveness, as well as for dispensary registration.

12. Reagent kit storage, transportation and operation conditions

Storage

Store HIV-1-test-Q reagent kit in the manufacturer's packaging at -18... 22°C during the entire kit shelf life, it can be stored at 2... 8°C up to 10 days.

It is not allowed to freeze/thaw HIV-1-test-Q reagent kit more than 10 times.

After opening, store under the same conditions as the reagents before opening.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation

Transport HIV-1-test-Q reagent kit by all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

Transport at -18... -22°C during the entire kit shelf life. Transportation is allowed at 2... 8°C up to 10 days, or at 15... 25°C up to 5 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, place a reagent kit in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared iced packs. The type, volume and quantity of iced packs placed in the thermocontainer with the transported reagent kits, as well as the thermocontainer volume are selected depending on the transportation duration and conditions.

Reagent kits transported in temperature regime violation cannot be used.

Shelf life

HIV-1-test-Q reagent kit shelf life is 12 months from the acceptance date of the manufacturer's QCD (Quality Control Dept), if all transportation, storage and operation conditions are met. A reagent kit with an expired shelf life cannot be used.

Shelf life of the opened kit components

12 months from the acceptance date of the manufacturer's QCD, if stored at -18... -22°C.

Shelf life of the kit components prepared for work

One hour under conditions that prevent the components from drying out, as well as extraneous biological material contamination.

13. Disposal

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

According to the classification of medical waste, the kits belong to Class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" are collected in reusable containers or disposable bags of any color (except yellow and red).

The remaining tubes and materials after the work are disposed of in accordance with the methodological recommendations "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" (MU 287-113).

Liquid components (reagents) are destroyed by draining into the sewer with preliminary reagent dilution with tap water 1:100 and removal of the remaining packaging as industrial or household waste.

HIV-1-test-Q reagent kit consumer packaging is subject to mechanical destruction with the residues removal as industrial or household waste.

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

14. Warranty, contacts

The manufacturer guarantees the reagent kit quality and safety during the shelf life in compliance with the kit 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, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072
Phone number: +7 (499) 705 03 75
www.testgene.com

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

Annex A

Designation	Document name
GOST ISO 14971-2011	Medical devices. Application of risk management to medical devices.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, kits, the test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R 51352-2013	In vitro diagnostic medical devices. Test methods.
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostic medical devices.
GOST R ISO 18113-1-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 2. In vitro diagnostic reagents for professional use.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling and information to be supplied. Part 1. General requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.