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March 27, 2023

INSTRUCTIONS FOR USE

Reagent kit for Varicella-Zoster Virus DNA detection by multiplex PCR-RT "VZV-test"

TS 21.20.23-069-97638376-2023

Version 1 dated March 27, 2023

Content

List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
VZV	Varicella-Zoster Virus
ICS	internal control sample
NC	negative control sample
PC	positive control sample
ESS	standard enterprise sample
SenC	sensitivity control sample
SC	specificity control sample
LRM	lyophilized reaction mixture

Introduction

The target analyte detected by VZV-test reagent kit is specific regions of VZV genomic DNA.

The scientific validity for choosing the target analyte lies in the specificity (DNA sequence uniqueness) in relation to the genome of the Varicella-Zoster Virus – herpes zoster.

Varicella Zoster virus (synonyms: VZV, Human alphaherpesvirus 3, HHV-3) is a species of DNA-containing viruses, from the genus *Varicellovirus* within the herpesvirus family (Herpesviridae). Like all herpesviruses, VZV is a double-stranded DNA virus with a 125,000-bp genome, comprising 71 genes.[1]

The virus is the causative agent of two diseases – chickenpox upon first contact with the virus and shingles upon the virus reactivation.

Chicken pox (Varicella). VZV enters the host through the upper respiratory tract, nasopharynx and in some cases through conjunctiva. It is most often a mild disease that does not require specific treatment, accompanied by fever, malaise, headache and itchy vesicular rash. Patients become contagious 1-2 days before the rash appears, infecting the contact ones by breathing, and remain contagious for the next 5-7 days of rash, secreting the virus from the skin vesicles.

The most common chickenpox complication is bacterial superinfection of the skin damaged by the rash, most often caused by Staphylococcus or Streptococcus. Less commonly, chickenpox can lead to viral pneumonia, which can have a mortality rate ranging from 10-30%, especially among immunocompromised adults and pregnant women. The most common neurological complications of primary VZV infection are encephalitis, acute cerebral ataxia, myelitis and meningitis.

[1] Study of the genetic diversity of the varicella zoster virus in certain regions of the Russian Federation using high-throughput sequencing / Nadtoka M. I., Lysenkov V. G., Agletdinov M. R. [and others] // Journal of Microbiology, Epidemiology and Immunobiology. – 2023. – Vol. 100, No. 5. – pp. 267-275.

In pregnant women, chickenpox is often complicated by viral pneumonia. VZV infection in early pregnancy increases the risk of developing birth defects in the fetus. Thus, the risk of developing congenital varicella syndrome with primary VZV infection in mothers between 13 to 20 weeks of pregnancy is 2%. This syndrome is characterized by scar lesions, limb hypoplasia, and eye defects. It is believed that the main lesions in congenital varicella syndrome occur due to intrauterine zoster-like reactivation of VZV. Nearly 30% of infants born with signs of congenital varicella syndrome die during the first few months of life.

Shingles (herpes zoster). In primary infection, VZV enters the sensory ganglia of the brain and spinal cord, as well as the ganglia of the autonomic nervous system, where it remains latent for life, retaining the ability to reactivate.

People over 60, patients receiving immunosuppressive therapy, corticosteroids, anti-TNF therapy, cancer patients, AIDS patients, and organ and bone marrow recipients after transplantation are at risk of virus reactivation.

The most common disease symptom is an unilateral itchy painful rash within 1-3 dermatomes, mainly of the thoracic region. Skin scarring may occur after the rash resolves.

In addition, there is a risk of scleritis, acute epithelial keratitis, uveitis, oculomotor nerve palsy, retinitis, optic neuritis, which can lead to vision impairment or even blindness. Virus DNA detection in the nodes of the celiac and nodular plexuses of the autonomic nervous system during VZV reactivation allows to assume the possibility of visceral organs damage. In some cases, especially in people with immunodeficiency conditions, VZV reactivation can cause generalized lesions such as granulomatous arteritis, leading to the development of hemorrhagic stroke, myelitis, meningitis and encephalitis.[2]

DNA samples isolated from clinical material are used for testing – rash scrapings, vesicular fluid, cerebrospinal fluid, conjunctival discharge, peripheral blood plasma, cord blood plasma, capillary blood,

[2] Chickenpox and shingles: features of morbidity and clinical manifestations / Lavrov V. F., Kazanova A. S., Kuzin S. N., Dubodelov D. V. // Epidemiology and infectious diseases. Current issues. - 2011. – No. 3. – P. 54.

amniotic fluid, saliva, oropharyngeal washes, oropharyngeal swabs.

Collect peripheral blood and cord blood plasma in a tube with 6% EDTA or 6% EDTA and gel.

Collect capillary blood in a tube with 6% EDTA.

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

Contraindications for use were not identified.

Indications for use: ATTENTION! A specialist doctor should determine a diagnosis and prescribe treatment.

A reagent kit is used in clinical laboratory diagnostics to study clinical material obtained from people with suspected infectious skin diseases, herpesvirus infection, regardless of the disease form and stage of all population groups, to determine a diagnosis.

There are no contraindications for use, except in cases when sampling cannot be carried out for medical reasons.

Population, demographic aspects of the medical device use: no population, demographic aspects of VZV-test reagent kit use were identified.

Sterility: the product is not sterile.

1. Intended use

Intended use: VZV-test reagent kit is designed for qualitative detection of a VZV genomic DNA specific region by multiplex allele-specific polymerase chain reaction with hybridization-fluorescence detection in a DNA sample isolated from clinical material (rash scrapings, vesicular fluid, cerebrospinal fluid, conjunctival discharge, peripheral blood plasma, cord blood plasma, capillary blood, amniotic fluid, saliva, oropharyngeal washes, oropharyngeal swabs) obtained from people with suspected infectious skin diseases, herpesvirus infection, regardless of the disease form and stage of all population groups.

Functional purpose: the obtained results can be used to diagnose infectious diseases caused by Varicella-Zoster Virus (VZV), regardless of the disease form and stage in all population groups.

A specialist doctor should determine a diagnosis and prescribe treatment based on clinical observations, medical history and epidemiological information in combination.

Potential consumers of a medical device:

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

2. Method principle

Method

Multiplex allele-specific real-time polymerase chain reaction with hybridization-fluorescence detection.

Test sample type

DNA samples isolated from clinical material are used for testing: rash scrapings, vesicular fluid, cerebrospinal fluid, conjunctival discharge, peripheral blood plasma, cord blood plasma, capillary blood, amniotic fluid, saliva, oropharyngeal washes, oropharyngeal swabs.

Collect peripheral blood and cord blood plasma in a tube with 6% EDTA or 6% EDTA and gel.

Collect capillary blood in a tube with 6% EDTA.

Detection principle

VZV DNA qualitative determination by multiplex PCR with real-time detection in a DNA sample includes four stages:

1. Sample preparation, DNA isolation from biomaterial;
2. PCR mixtures preparation;
3. DNA PCR amplification with hybridization-fluorescence detection of amplification products in real time;
4. Result interpretation.

DNA samples undergo amplification reactions of DNA regions using specific primers in a PCR reaction buffer.

PCR Buffer contains all the basic reagents, including a thermostable hot start DNA polymerase, dNTP, uracil-DNA glycosylase and an optimized buffer. The presence of the enzyme uracil-DNA glycosidase prevents false positive results in case of contamination with amplification products, while the enzyme is completely inactivated during the first DNA denaturation cycle, it does not prevent a current reaction product amplification.

The Primer Mix 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 are separated, and the fluorescence intensity increases. This allows the specific amplification product accumulation to be recorded by measuring the fluorescent signal intensity in real time.

The kit contains reagents for the multiplex detection of VZV highly specific DNA region and DNA included in the internal control sample (ICS) (Table 1). ICS allows to evaluate DNA isolation efficiency and the possibility of the inhibitors presence in the sample, which can lead to false negative results.

Table 1 – Test targets

Channel corresponding to the fluorophore	
FAM/Green	**HEX/Yellow**
VZV DNA	DNA ICS

Method limitations

A possible reason for obtaining a false positive result is contamination at the DNA isolation or multiplex PCR reaction stage. A false positive result can be detected using a negative control sample.

A reagent kit cannot be used 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.

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

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

The multiplex PCR reaction time is 60-80 minutes (excluding sample preparation), depending on the used cycler.

3. Reagent kit components

The reagent kit is designed in four configuration forms:

Configuration form 1, VZV-test – includes VZV-test reagent kit for PCR;

Configuration form 2, VZV-test-extra – includes VZV-test reagent kit for PCR and DNA-extra-VZV reagent kit for DNA isolation;

Configuration form 3, VZV-test-Lyo – includes VZV-test-Lyo reagent kit for PCR;

Configuration form 4, VZV-test-extra-Lyo – includes VZV-test-Lyo reagent kit for PCR and DNA-extra-VZV reagent kit for DNA isolation.

VZV-test reagent kit delivery package includes:

- Reagent kit (configuration form 1, 2, 3 or 4) – 1 pc.,
- Instructions for use – 1 pc.,
- Quality certificate – 1 pc. per batch.

It is allowed to supply one instruction for use for several jointly supplied products. The instructions for use and the product certificate are included in the transport packaging per batch.

Number of test samples

For all forms of configuration - the reagent kit is designed for 96 reactions, which corresponds to detection of 94 test samples, negative and positive samples during a single run of a 96-well cycler or 32 single test sample detections with negative and positive control samples in each test.

Table 2 – Components of the reagent kit, configuration form 1 – VZV-test

Item	Reagent name	Description	Quantity, volume
1	PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2	Primer Mix	Transparent liquid with a pink shade	1 tube, 480 µl
3	ICS	Transparent colorless liquid	1 tube, 960 µl
4	PC	Transparent colorless liquid	1 tube, 480 µl
5	NC	Transparent colorless liquid	1 tube, 1800 µl

Table 3 – Components of the reagent kit, configuration form 2 – VZV-test-extra

Item	Reagent name	Description	Quantity, volume
VZV-test reagent kit for PCR			
1	PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2	Primer Mix	Transparent liquid with a pink shade	1 tube, 480 µl
3	ICS	Transparent colorless liquid	1 tube, 960 µl
4	PC	Transparent colorless liquid	1 tube, 480 µl
5	NC	Transparent colorless liquid	1 tube, 1800 µl
DNA-extra-VZV reagent kit for DNA isolation			
1	Binding Buffer	Transparent colorless liquid, may have a yellow shade	1 bottle (48 ml)
2	Magnetic Beads	Brown suspension	1 tube, (960 µl)
3	Wash solution No. 1	Transparent colorless liquid	1 bottle (68 ml)
4	Wash solution No. 2	Transparent colorless liquid	2 bottles (68 ml each)
5	Eluent	Transparent colorless liquid	1 bottle (21 ml)

Table 4 – Components of the reagent kit, configuration form 3 – VZV-test-Lyo

Item	Reagent name	Description	Quantity, volume
1	LRM	Dry amorphous porous mass of white or light pink color	96 tubes connected with bridges in a plate
2	PC	Dry amorphous porous mass of white color	1 bottle (lyophilizate)
3	ICS	Dry amorphous porous mass of white color	1 bottle (lyophilizate)
4	Reconstitution solution for PC	Transparent colorless liquid	1 tube, 704 µl
5	Reconstitution solution for ICS	Transparent colorless liquid	1 tube, 960 µl
6	NC	Transparent colorless liquid	2 tubes, 1800 µl
7.	Sealing film for a PCR plate	A transparent film designed to seal PCR plates and prevent sample loss during amplification	2 pcs.

Table 5 – Components of the reagent kit, configuration form 4 – VZV-test-extra-Lyo

Item	Reagent name	Description	Quantity, volume
VZV-test-Lyo reagent kit for PCR			
1	LRM	Dry amorphous porous mass of white or light pink color	96 tubes connected with bridges in a plate
2	PC	Dry amorphous porous mass of white color	1 bottle (lyophilizate)
3	ICS	Dry amorphous porous mass of white color	1 bottle (lyophilizate)
4	Reconstitution solution for PC	Transparent colorless liquid	1 tube, 704 µl
5	Reconstitution solution for ICS	Transparent colorless liquid	1 tube, 960 µl
6	NC	Transparent colorless liquid	2 tubes, 1800 µl
7.	Sealing film for a PCR plate	A transparent film designed to seal PCR plates and prevent sample loss during amplification	2 pcs.
DNA-extra-VZV reagent kit for DNA isolation			
1	Binding Buffer	Transparent colorless liquid, may have a yellow shade	1 bottle (48 ml)
2	Magnetic Beads	Brown suspension	1 tube, (960 µl)
3	Wash solution No. 1	Transparent colorless liquid	1 bottle (68 ml)
4	Wash solution No. 2	Transparent colorless liquid	2 bottles (68 ml each)
5	Eluent	Transparent colorless liquid	1 bottle (21 ml)

VZV-test PCR reagent kit components description

PCR Buffer is ready for use and contains all the basic reagents, including thermostable hot start DNA polymerase, deoxynucleotide triphosphates, including dUTP, uracil-DNA glycosylase (UDG), and an optimized buffer.

Primer Mix is ready for use and contains a multiplex mixture of primers and probes:

1. Primers and a probe to the VZV genomic DNA specific region. Detection is carried out in the FAM/Green channel.
2. Primers and ICS probe. Detection is carried out in the HEX/Yellow channel.

Internal control sample (ICS) is ready for use and is a plasmid DNA with a synthetic insertion of a specific ICS DNA fragment.

Positive control sample (PC) is ready for use and is a mixture of plasmid DNA containing synthetic insertions of amplified fragments of Varicella Zoster virus genomic DNA and internal control sample DNA.

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

VZV-test-Lyo reagent kit for PCR components description

Lyophilized reaction mixture (LRM) is a lyophilized freeze-dried buffer containing all the basic reagents, including a thermostable hot start DNA polymerase, deoxynucleotide triphosphates (including dUTP), uracil-DNA glycosylase (UDG), an optimized buffer and cryoprotectant mixture. This mixture also contains primers and probes designed to detect specific targets – see Table 1.

Positive control sample (PC) is ready for use and is a lyophilized mixture of plasmid DNA containing synthetic insertions of amplified fragments of Varicella Zoster virus genomic DNA and internal control sample DNA.

Negative control sample (NCO) is ready for use and is DNase/RNase-free deionized water.

Internal control sample (ICS) is ready for use and is a lyophilized plasmid DNA preparation with a synthetic insertion of a specific ICS DNA fragment.

Reconstitution solution for PC is DNase/RNase-free deionized water.

Reconstitution solution for ICS is DNase/RNase-free deionized water.

DNA-extra-VZV reagent kit for DNA isolation components description

Binding Buffer is ready for use and includes components for biomaterial lysing and DNA binding to magnetic beads surface.

Magnetic bead solution is ready for use and is a magnetic bead suspension.

Wash solution No. 1 is ready for use and includes components for washing DNA from biomaterial residues.

Wash solution No. 2 is ready for use and includes components for washing biomaterial residues and Wash Solution No. 1.

Eluent is ready for use and includes components for purified DNA desorption from the magnetic beads surface.

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

4. Reagent kit characteristics

4.1. Technical and functional characteristics

Table 6 – VZV-test reagent kit, configuration form 1 – VZV-test characteristics

Indicator	Characteristics and standards
VZV-test reagent kit for PCR	
1. Technical characteristics	
1.1. Appearance	
PCR Buffer	Transparent colorless liquid
Primer Mix	Transparent liquid with a pink shade
ICS	Transparent colorless liquid
PC	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to clause 1.4 TS 21.20.23-069-97638376-2023
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023

2. Functional characteristics	
Positive result with PC	Fluorescence signal growth recorded in a tube with PC in the FAM channel Ct ≤ 28 and in the HEX channel Ct ≤ 28
Negative result with NC	In a tube with NC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35
Reaction with ICS	Fluorescence signal growth recorded in tubes with ICS in the FAM channel Ct is not indicated (that is, there is no fluorescence accumulation curve) and in the HEX channel Ct ≤ 28
Reaction with ESS-SC	In a tube with ESS-SC in the FAM and NEX channels Ct is not indicated (i.e. there is no fluorescence accumulation curve)
Reaction with ESS-SenC	In tubes with ESS-SenC in all repetitions (at least 4), in the FAM channel Ct ≤ 35, and the HEX channel Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35

Table 7 – VZV-test reagent kit, configuration form 2 – VZV-test-extra characteristics

Indicator	Characteristics and standards
VZV-test reagent kit for PCR	
1. Technical characteristics	
1.1. Appearance	
PCR Buffer	Transparent colorless liquid
Primer Mix	Transparent liquid with a pink shade
ICS	Transparent colorless liquid
PC	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to clause 1.4 TS 21.20.23-069-97638376-2023
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023
2. Functional characteristics	
Positive result with PC	Fluorescence signal growth recorded in a tube with PC in the FAM channel Ct ≤ 28 and in the HEX channel Ct ≤ 28
Negative result with NC	In a tube with NC in the FAM and NEX channels Ct is not indicated (i.e. there is no fluorescence accumulation curve) or Ct ≥ 35

Reaction with ICS	Fluorescence signal growth recorded in tubes with ICS in the FAM channel Ct is not indicated (that is, there is no fluorescence accumulation curve) and in the HEX channel Ct ≤ 28
Reaction with ESS-SC	In a tube with ESS-SC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve)
Reaction with ESS-SenC	In tubes with ESS-SenC in all repetitions (at least 4), in the FAM channel Ct ≤ 35, and the HEX channel Ct is not indicated (i.e., there is no fluorescence accumulation curve) or Ct ≥ 35
Indicator	**Characteristics and standards**
DNA-extra-VZV reagent kit for DNA isolation	
1. Technical characteristics	
1.1. Appearance	
Binding Buffer	Transparent colorless liquid, may have a yellow shade
Magnetic Beads, MB	Brown suspension
Wash solution No. 1	Transparent colorless liquid
Wash solution No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
1.2. Physical-chemical parameters	
Hydrogen ion concentration indicators, pH	
Binding Buffer	min 7.0 pH, max 9.0 pH
Wash solution No. 1	min 6.0 pH, max 8.0 pH
Wash solution No. 2	min 6.0 pH, max 8.0 pH
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023
2. Functional characteristics	
2.2 Absence of kit components contamination with extraneous DNA/RNA	Negative result with NC in the control PCR in the FAM and HEX channels
2.3. DNA suitability for PCR	When testing an isolation control sample that has passed the DNA isolation stage using DNA-Extra-VZV kit, in FAM channels Ct no more than 30 cycles and Δ Ct value between the isolation control sample that has passed the isolation stage and isolation control sample without isolation in the FAM channels is no more than 2 cycles (in all duplicates)

Table 8 – VZV-test reagent kit, configuration form 3 – VZV-test-Lyo characteristics

Indicator	Characteristics and standards
VZV-test-Lyo reagent kit for PCR	
1. Technical characteristics	
1.1. Appearance	
LRM	Dry amorphous porous mass of white or light pink color
PC	Dry amorphous porous mass of white color
NC	Transparent colorless liquid
ICS	Dry amorphous porous mass of white color
Reconstitution solution for PC	Transparent colorless liquid
Reconstitution solution for ICS	Transparent colorless liquid
Sealing film for a PCR plate	A transparent film designed to seal PCR plates and prevent sample loss during amplification
1.2. Completeness	According to clause 1.4 TS 21.20.23-069-97638376-2023
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023
2. Functional characteristics	
Positive result with PC	Fluorescence signal growth registered in a tube with PC in the FAM channel Ct ≤ 28 and in the HEX channel Ct ≤ 28
Negative result with NC	In a tube with NC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35
Reaction with ICS	Fluorescence signal growth recorded in tubes with ICS in the FAM channel Ct is not indicated (that is, there is no fluorescence accumulation curve) and in the HEX channel Ct ≤ 28
Reaction with ESS-SC	In a tube with ESS-SC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve)
Reaction with ESS-SenC	In tubes with ECC-SenC in all repetitions (at least 4), in the FAM channel Ct ≤ 35, and in the HEX channel Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35

Table 9 – VZV-test reagent kit, configuration form 4 – VZV-test-extra-Lyo characteristics

Indicator	Characteristics and standards
VZV-test-Lyo reagent kit for PCR	
1. Technical characteristics	
1.1. Appearance	
LRM	Dry amorphous porous mass of white or light pink color
PC	Dry amorphous porous mass of white color
NC	Transparent colorless liquid
ICS	Dry amorphous porous mass of white color
Reconstitution solution for PC	Transparent colorless liquid
Reconstitution solution for ICS	Transparent colorless liquid
Sealing film for a PCR plate	A transparent film designed to seal PCR plates and prevent sample loss during amplification
1.2. Completeness	According to clause 1.4 TS 21.20.23-069-97638376-2023
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023
2. Functional characteristics	
Positive result with PC	Fluorescence signal growth registered in a tube with PC in the FAM channel Ct ≤ 28 and in the HEX channel Ct ≤ 28
Negative result with NC	In a tube with NC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35
Reaction with ICS	Fluorescence signal growth recorded in tubes with ICS in the FAM channel Ct is not indicated (that is, there is no fluorescence accumulation curve) and in the HEX channel Ct ≤ 28
Reaction with ESS-SC	In a tube with ESS-SC in the FAM and NEX channels Ct is not indicated (that is, there is no fluorescence accumulation curve)
Reaction with ESS-SenC	In tubes with ECC-SenC in all repetitions (at least 4), in the FAM channel Ct ≤ 35, and in the HEX channel Ct is not indicated (that is, there is no fluorescence accumulation curve) or Ct ≥ 35

Table 9 (continuation)

Indicator	Characteristics and standards
DNA-extra-VZV reagent kit for DNA isolation	
1. Technical characteristics	
1.1. Appearance	
Binding Buffer	Transparent colorless liquid, may have a yellow shade
Magnetic Beads, MB	Brown suspension
Wash solution No. 1	Transparent colorless liquid
Wash solution No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
Indicator	**Characteristics and standards**
1.2. Physical-chemical parameters	
Hydrogen ion concentration indicators, pH	
Binding Buffer	min 7.0 pH, max 9.0 pH
Wash solution No. 1	min 6.0 pH, max 8.0 pH
Wash solution No. 2	min 6.0 pH, max 8.0 pH
1.3. Labelling	According to clause 1.5 TS 21.20.23-069-97638376-2023
1.4. Packaging	According to clause 1.6 TS 21.20.23-069-97638376-2023
2. Functional characteristics	
2.2. Absence of kit components contamination with extraneous DNA	Negative result with NC in the control PCR in the FAM channel
2.3. DNA suitability for PCR	When testing an isolation control sample that has passed the DNA isolation stage using DNA-Extra-VZV kit, in FAM channels Ct is no more than 30 cycles and Δ Ct value between the isolation control sample that has passed the isolation stage and isolation control sample without isolation in the FAM channel is no more than 2 cycles (in all duplicates).

Note: The control PCR is performed using a standard enterprise sample of ESS-Extra-VZV kit.

When performing control PCR, deionized DNase/RNase-free water is used as a negative control sample (NC) included in ESS-Extra-VZV kit.

A plasmid DNA solution containing a specific fragment of human genomic DNA is used as an isolation control sample included in the ESS-Extra-VZV kit.

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

4.2. Analytical efficiency characteristics

4.2.1. Analytical sensitivity

It is specific to Varicella Zoster virus DNA.

The absence of non-specific positive amplification results in the presence of the following organisms and viruses in the DNA sample was shown: Human alphaherpesvirus 1, Human alphaherpesvirus 2, Epstein-Barr virus, Cytomegalovirus, Human betaherpesvirus 6A, Human betaherpesvirus 6B, Measles morbillivirus, Rubella virus, *Streptococcus anginosus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Toxoplasma gondii*.

4.2.2. Interfering substances effect evaluation

The effect of potentially interfering substances on VZV-test reagent kit performance was tested for potentially interfering substances, which may occur during VZV-test reagent kit normal use, and may affect the kit's ability to provide accurate results.

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

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

2) substances found in specific sample types - in this case, clinical sample contamination with blood hemoglobin can inhibit PCR in case of insufficient purification during the DNA isolation procedure.

The studied concentrations of interfering substances are shown in Table 10.

Table 10 – Conducted assay results analysis.

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	100 mg/ml
Mucin	2.3 mg/ml
Exogenous interfering substances	
Therapeutic and prophylactic drugs	
Chlorhexidine	0.05 g/ml
Miramistin (benzyldimethyl[3 (myristoylamino) propyl]ammonium chloride monohydrate)	0.1 μg/ml

Based on the assay results, these substances do not have an interfering effect on the kit operation and do not lead to PCR inhibition at concentrations not exceeding the permissible ones.

To reduce PCR inhibitors amount, it is required to follow clinical material sampling rules.

Limitations on the test material use:

- the test material cannot be used in case of storage and transportation conditions violation (temperature, duration, multiple freezing and thawing);
- it is not allowed to use samples contaminated with extraneous biological material.

4.2.3. Analytical sensitivity: Limit of detection (LOD)

In accordance with GOST R 51352-2013 and taking into account the international recommendations of CLSI EP-17A2, the limit of detection (LOD) was determined by the dilution analysis method of the WHO International Standard in the range of the estimated detection limit: 100, 150, 250, 300, 350, 450, 500 copies/ml (2.10; 2.27; 2.49; 2.57; 2.64; 2.75; 2.80 log10 IU/ml) and 100, 250, 350, 500, 600, 850, 1000 copies/ml (2.10; 2.50; 2.64; 2.80; 2.91; 3.03; 3.10 log10 IU/ml):

1st WHO International Standard for VZV NAT Assays NIBSC Code: 19/164.

Based on the ECBS review of study report WHO/BS/2021.2405 19/164 was established as the 1st WHO international standard for Varicella-Zoster virus DNA for NAT-based assays with an assigned measurement unit 7.0 log10 IU/vial (6.8 log10 copies/ml).

For Varicella-Zoster virus DNA qualitative detection, a reagent kit registered in the established manner was used - reagent kit for Varicella-Zoster virus (VZV) DNA detection in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection AmpliSens® VZV-FL according to TS 9398-183-01897593-2011 (RC FSR No. 2012/13619 dated March 27, 2019)

Varicella Zoster virus DNA concentration in the tested samples was determined using QX200 Droplet Digital PCR System (RC No. RZN 2022/17351). k

Based on VZV-test reagent kit study results, the limit of Varicella Zoster virus DNA detection in samples with 95% detection rate for each cycler is:

Table 11 – Varicella Zoster virus DNA limit of detection in samples

Configuration form (CF) of VZV-test reagent kit	**DNA isolation reagent kit**	**Used cycler**	**VZV DNA concentration, IU/ml (LOD) with 95% confidence probability**	**Confidence interval with 95% confidence probability**
CF 1 - VZV-test	DNA-Fast	DTprime	1093.22	1087.85-1098.59
		CFX 96	1174.78	1169.41-1180.15
		Rotor-Gene Q	1247.21	1241.84-1252.58
		QuantStudio 5	1155.85	1150.48-1161.22
		FLUORITE	1293.62	1288.25-1298.99
	NA-Extra	DTprime	574.24	568.87-579.61
		CFX 96	562.40	557.03-567.77
		Rotor-Gene Q	554.39	549.02-559.76
		QuantStudio 5	567.47	562.10-572.84
		FLUORITE	539.94	534.57-545.31
CF 2 - VZV-test-extra	DNA-extra-VZV, included in VZV-test reagent kit, CF 2	DTprime	558.80	553.43-564.17
		CFX 96	578.06	572.69-583.43
		Rotor-Gene Q	550.20	544.83-555.57
		QuantStudio 5	555.46	550.09-560.83
		FLUORITE	616.37	611.00-621.74
CF 3 - VZV-test-Lyo	DNA-Fast	DTprime	1104.97	1099.60-1110.34
		CFX 96	1262.14	1256.77-1267.51
		Rotor-Gene Q	1154.22	1148.85-1159.59
		QuantStudio 5	1020.39	1015.02-1025.76
		FLUORITE	1116.87	1111.50-1122.24
	NA-Extra	DTprime	604.78	599.41-610.15
		CFX 96	562.05	556.68-567.42
		Rotor-Gene Q	625.19	619.82-630.56
		QuantStudio 5	545.05	539.68-550.42
		FLUORITE	571.55	566.18-576.92
CF 4 - VZV-test-extra-Lyo	DNA-extra-VZV, included in VZV-test reagent kit, CF 4	DTprime	627.87	622.50-633.24
		CFX 96	622.48	617.11-627.85
		Rotor-Gene Q	573.46	568.09-578.83
		QuantStudio 5	551.20	545.83-556.57
		FLUORITE	605.55	600.18-610.92

4.2.4. Precision under repeatability conditions

To evaluate precision under repeatability conditions, a positive control sample, an internal control sample were tested in 10 repetitions.

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

The assay results showed that the coefficient of variation under the kit repeatability conditions does not exceed 3%.

4.2.5. Precision under reproducibility conditions

The test system reproducibility is evaluated similarly to the calculation of precision under repeatability conditions (Section 4.2.3.), 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).

When performing precision assay under reproducibility conditions, complete intra-assay, inter-assay and inter-series reproducibility was observed, the coefficient of variation does not exceed 5%.

4.2.6. Metrological traceability

Metrological traceability of calibration and the assigned value of the end–user calibrators – PC, included in VZV-test reagent kit, and the used calibrators ESS-SenC, ESS-SC in accordance with GOST R ISO 17511-2022.

The calibration hierarchy of PC, ESS-SenC was carried out with an internationally accepted calibrator, which determines the measured value (clause 5.5 GOST R ISO 17511-2022):

1st WHO International Standard for VZV NAT Assays NIBSC Code: 19/164

The general calibration hierarchy with indicated measurement uncertainty at each stage is shown in Tables 12 and 13.

Table 12 – Calibration hierarchy results

Analyte	VZV DNA		
Sample type	Internationally accepted calibrator	Used calibrator	End user IVD medical device calibrator
Sample	1st WHO International Standard for VZV NAT Assays NIBSC Code: 19/164	ESS-SenC	PC
Measurement uncertainty	$u_{m.3} = 0.36$	$u_{m.3} = 0.36$	$u_{p.5} = 0.06$ $u_{cal} = 0.37$

Table 13 – Combined standard uncertainty and combined extended uncertainty

Combined standard uncertainty		**Combined expanded uncertainty**	
$u(y) = 0.48$	rash scrapings	$U(y) = 0.96$	rash scrapings
$u(y) = 0.54$	vesicular fluid	$U(y) = 1.08$	vesicular fluid
$u(y) = 0.43$	cerebrospinal fluid	$U(y) = 0.86$	cerebrospinal fluid
$u(y) = 0.43$	conjunctival discharge	$U(y) = 0.86$	conjunctival discharge
$u(y) = 0.41$	peripheral blood plasma	$U(y) = 0.82$	peripheral blood plasma
$u(y) = 0.52$	cord blood plasma	$U(y) = 1.04$	cord blood plasma
$u(y) = 0.47$	capillary blood	$U(y) = 0.94$	capillary blood
$u(y) = 0.49$	amniotic fluid	$U(y) = 0.98$	amniotic fluid
$u(y) = 0.51$	saliva	$U(y) = 1.02$	saliva
$u(y) = 0.51$	oropharyngeal washes	$U(y) = 1.02$	oropharyngeal washes
$u(y) = 0.49$	oropharyngeal swabs	$U(y) = 0.98$	oropharyngeal swabs

The assigned concentration of the PC end-user calibrator is $8x10^5$ copies/ml, of the used calibrators ESS-SenC is 500 copies/ml.

In accordance with clause 4.7.1 (c) GOST R ISO 17511-2022, the combined standard measurement uncertainty of the value assigned by the end user IVD medical device calibrator (PC) u_{cal} does not exceed the permissible ratio *Umax(y)* of specification for the IVD medical device, taking into account a coverage factor *k* (*k* = 2, for an approximate 95% confidence probability):

Table 14 – Evaluation of combined standard measurement uncertainty.

Evaluation of combined standard measurement uncertainty of VZV-test reagent kit in relation to VZV DNA	**Clinical material**
$u_{cal} = 0.96 \leq \frac{1}{2} Umax(y) = 1$	rash scrapings
$u_{cal} = 1.08 \leq \frac{1}{2} Umax(y) = 1$	vesicular fluid
$u_{cal} = 0.86 \leq \frac{1}{2} Umax(y) = 1$	cerebrospinal fluid
$u_{cal} = 0.86 \leq \frac{1}{2} Umax(y) = 1$	conjunctival discharge
$u_{cal} = 0.82 \leq \frac{1}{2} Umax(y) = 1$	peripheral blood plasma
$u_{cal} = 1.04 \leq \frac{1}{2} Umax(y) = 1$	cord blood plasma
$u_{cal} = 0.94 \leq \frac{1}{2} Umax(y) = 1$	capillary blood
$u_{cal} = 0.98 \leq \frac{1}{2} Umax(y) = 1$	amniotic fluid
$u_{cal} = 1.02 \leq \frac{1}{2} Umax(y) = 1$	saliva
$u_{cal} = 1.02 \leq \frac{1}{2} Umax(y) = 1$	oropharyngeal washes
$u_{cal} = 0.98 \leq \frac{1}{2} Umax(y) = 1$	oropharyngeal swabs

And in accordance with clause 4.1 (c) GOST R ISO 17511-2022, the estimated combined expanded measurement uncertainty U(y) does not exceed the maximum permissible measurement uncertainty Umax(y):

Table 15 – Evaluation of combined expanded measurement uncertainty.

Evaluation of combined expanded measurement uncertainty of VZV-test reagent kit in relation to VZV DNA	**Clinical material**
U(y)=0.96 ≤ Umax(y)=2	rash scrapings
U(y)=1.08 ≤ Umax(y)=2	vesicular fluid
U(y)=0.86 ≤ Umax(y)=2	cerebrospinal fluid
U(y)=0.86 ≤ Umax(y)=2	conjunctival discharge
U(y)=0.82 ≤ Umax(y)=2	peripheral blood plasma
U(y)=1.04 ≤ Umax(y)=2	cord blood plasma
U(y)=0.94 ≤ Umax(y)=2	capillary blood
U(y)=0.98 ≤ Umax(y)=2	amniotic fluid
U(y)=1.02 ≤ Umax(y)=2	saliva
U(y)=1.02 ≤ Umax(y)=2	oropharyngeal washes
U(y)=0.98 ≤ Umax(y)=2	oropharyngeal swabs

The calibration hierarchy of ESS-SC was carried out with the primary reference measurement procedure, determining the measured value (clause 5.3 GOST R ISO 17511-2022).

The general calibration hierarchy with indicated measurement uncertainty at each stage is shown in Table 16.

Table 16 – General calibration hierarchy

Analyte	human genomic DNA isolated from the U937 cell line	
Sample	Secondary SS - human genomic DNA isolated from the U937 cell line (manufactured by SibEnzyme LLC, Russia)	ESS-SC
Sample type	Secondary SS	Used calibrator
Measurement uncertainty	$u_{m.3} = 0.78$	$u_{p.4} = 0.1$
Combined standard uncertainty	$u(y) = 0.78$	
Combined expanded uncertainty	$U(y) = 1.56$	

The attributed concentration of the used calibrator **ESS-SC -** 1000 copies/ml.

4.3. Clinical efficiency characteristics

140 clinical material samples (16 - rash scrapings, 17 - vesicular fluid, 4 - cerebrospinal fluid, 15 - conjunctival discharge, 20 - peripheral blood plasma, 4 - umbilical cord blood plasma, 17 - capillary blood, 4 - amniotic fluid, 14 - saliva, 14 - oropharyngeal washes, 15 - oropharyngeal swabs) were used for clinical assays. These samples were collected from patients with suspected infectious diseases caused by the varicella zoster virus (VZV), which were obtained from a bank of residual aliquots formed during routine medical and diagnostic practice.

This number of samples was taken in accordance with the recommendations of GOST R 51352-2013 and considering the recommendations of the International Guideline CLSI EP09-A3.

Each clinical sample was tested in two series using VZV-test reagent kit and the obtained data were compared with the results obtained by Federal State Budgetary Educational Institution of Higher Education "Samara State Medical University" of the Ministry of Healthcare of the Russian Federation using registered medical devices:

- Reagent kit for Varicella-Zoster virus (VZV) DNA determination in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection AmpliSens® VZV-FL according to TS 9398-183-01897593-2011, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, registration certificate No. FSR 2012/13619 dated March 27, 2019;

- Reagent kit for Varicella-zoster virus (VZV) DNA detection by real-time PCR AmpliPrime® VZV according to TS 21.20.23-170-09286667-2022", manufactured by NextBio LLC, Russia, registration certificate No. RZN 2023/20041 dated April 10, 2023.

The results matched, indicating that the medical device was functioning correctly.

To conduct PCR study using VZV-test reagent kit, configuration forms 1 and 2, cyclers recommended by the manufacturer of the tested reagent kit were used:

- DTprime detecting cycler, NPO DNA-Technology LLC, Russia (Registration certificate No. FSR 2011/10229 dated March 3, 2011);

- Rotor-Gene-Q detecting cycler, Qiagen, Germany (Registration certificate No. FSZ 2010/07595 dated August 10, 2010);

- QuantStudio 5 detecting cycler, Thermo Fisher Scientific, USA

(Registration certificate No. RZN 2019/8446 dated June 6, 2019);

- CFX96 detecting cycler, BioRad, USA (Registration certificate No. FSZ 2008/03399 dated June 21, 2016);

- FLUORITE cycler, Xian TianLong Science and Technology Co, China (Registration Certificate No. RZN 2022/16415 dated April 4, 2024).

To conduct PCR study using VZV-test reagent kit, configuration forms 3 and 4, cyclers recommended by the manufacturer of the tested reagent kit we used:

- DTprime detecting cycler, NPO DNA-Technology LLC, Russia (Registration certificate No. FSR 2011/10229 dated March 3, 2011);
- QuantStudio 5 detecting cycler, Thermo Fisher Scientific, USA (Registration certificate No. RZN 2019/8446 dated June 6, 2019);
- CFX96 detecting cycler, BioRad, USA (Registration certificate No. FSZ 2008/03399 dated June 21, 2016);
- FLUORITE cycler, Xian TianLong Science and Technology Co, China (Registration Certificate No. RZN 2022/16415 dated April 4, 2024).

Results reproducibility for all used cyclers is 100%.

Confidence intervals (CI) of diagnostic characteristics were calculated using the Clopper and Pearson Confidence Interval (Clopper, C., & Pearson, E. (1934)).

The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika, 26(4), 404-413. doi:10.2307/2331986). The diagnostic characteristics of the test kit were calculated with 95% confidence probability.

Table 17 – Study results of clinical material samples diagnostic characteristics

Test material type	Number of observations with positive samples	Number of observations with negative samples	Diagnostic specificity with 95% confidence probability	Diagnostic specificity with 95% confidence probability
Rash scrapings	32	248	100% (95% CI:89.11%-100%)	100% (95% CI:98.52%-100%)
Vesicular fluid	34	246	100% (95% CI:89.72%- 100%)	100% (95% CI:98.51%-100%)
Cerebrospinal fluid	8	272	100% (95% CI:63.06%- 100%)	100% (95% CI:98.65%- 100%)
Conjunctival discharge	30	250	100% (95% CI:88.43%- 100%)	100% (95% CI:98.54%-100%)
Peripheral blood plasma	40	240	100% (95% CI:91.19%- 100%)	100% (95% CI:98.47%-100%)
Cord blood plasma	8	272	100% (95% CI:63.06%- 100%)	100% (95% CI:98.65%-100%)
Capillary blood	34	246	100% (95% CI:89.72%- 100%)	100% (95% CI:98.51%-100%)
Amniotic fluid	8	272	100% (95% CI:63.06%- 100%)	100% (95% CI:98.65%-100%)
Saliva	28	252	100% (95% CI:87.66%-100%)	100% (95% CI:98.55%-100%)
Oropharyngeal washes	28	252	100% (95% CI:87.66%-100%)	100% (95% CI:98.55%-100%)
Oropharyngeal swabs	30	250	100% (95% CI:88.43%-100%)	100% (95% CI:98.54%-100%)

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. loss of functional properties of the reagents included in the kit due to transportation, storage or operation under inappropriate conditions;
2. testing using a poor-quality DNA sample (low concentration and/or poor purification);
3. clinical material contamination with inhibitory substances in concentrations exceeding permissible levels;
4. contamination of reaction mixtures and DNA test samples with contents from a PC tube or with PCR products;
5. failure to comply with the requirements for sample preparation, testing and disposal due to unqualified personnel work;
6. use of an unsuitable kit (use after the expiry or in case of damaged packaging).

No risks identified in the unacceptable risk zone.

The cumulative residual risk of using a medical device "Reagent kit for Varicella-Zoster Virus DNA detection by multiplex PCR-RT "VZV-test" according to TS 21.20.23-069-97638376-2023" is acceptable, the benefit of its use exceeds the risk.

6. Safety precautions

The class, depending on the potential risk of use - 2b - in accordance with the medical devices nomenclature classification approved by the order of the Ministry of Health of the Russian Federation dated 06.06.2012 N 4n.

All components and reagents included in VZV-test 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 VZV-test kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in VZV-test kit are non-toxic, as they are prepared by mixing individual non-toxic components.

Work with material infected or suspected of being infected is carried out in accordance with the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of

infectious diseases".

It is required 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) essays of clinical material in compliance with sanitary and epidemiological rules SanPiN 2.1.3684-21 dated January 28, 2021 "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 MU 287-113, 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 dated January 28, 2021 "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";

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable 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 use, according to the Instructions for Use;

- only specially trained personnel is allowed to work with the kit (a specialist with higher medical education who has been trained in licensed specialization courses for working with PBA of pathogenicity groups III-IV and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

- do not use the kit after the expiry date;

- avoid contact with skin, eyes and mucous membranes. 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 DNA-extra-VZV reagent kit for DNA isolation is carried out in working area 2 (for reaction preparation) (MU 1.3.2569-09).

Work with VZV-test and VZV-test-extra-Lyo reagent kit for PCR is carried out in working area 3 (for reaction preparation) (MU 1.3.2569-09).

Required equipment for DNA isolation (configuration forms 2 and 4):

1. Class II sterile laminar biological (microbiological) safety cabinet;
2. Thermostat for 1.5 ml Eppendorf type tubes, maintaining a temperature from 25 to 100°C;
3. Microcentrifuge-vortex for 1.5 ml Eppendorf type tubes;
4. Centrifuge for micro tubes, with RCF higher than 16,000 g;
5. Variable volume dispensers that allow to take liquid volumes of 0.5-10 µl, 2-20 µl, 20-200 µl, 200-1000 µl;
6. Refrigerator from from 2 to 8°C,
7. Freezer from -40 to -2°C;

Additional equipment for DNA isolation (configuration forms 2 and 4):

1. KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification, manufactured by Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022;
2. Pipetting sample preparation workstation Tecan Freedom EVO® series, TECAN, Switzerland, RC No. FSZ 2008/03047 dated July 4, 2016;

3. Multi-channel automatic variable volume dispensers (e.g. Eppendorf, Germany) or pipetting sample preparation workstation (Tecan Freedom EVO® series, TECAN, Switzerland, Austria or similar) can be used to introduce reagents into 96DW deep-well plates,

4. An electric laboratory aspirator with a trap flask.

Equipment required for multiplex PCR:

1. Class II and III biological safety box (e.g., microbial safety boxes BMB-II-Laminar-C according to TS 32.50.50-010-51495026-2020, manufactured by Laminar Systems CC, RC No. FSR 2012/13259 dated July 29, 2021 or a box for clean operations work DNA/RNA UV-Cleaner Box UVC/T-M-AR, Biosan, Latvia, RC No. RZN 2023/19369 dated January 18, 2023);

2. Vortex (e.g., Microspin 12 high-speed mini-centrifuge, BIOSAN SIA, Latvia, RC No. FSZ 2011/10116 dated July 11,2011 or CM-70M centrifuge-mixer, manufactured by ELMI SIA, Latvia, RC No. RZN 2016/4616 dated May 31, 2023);

3. Centrifuge for PCR plates (e.g., Refrigerated Laboratory Centrifuge LMC-4200R, BIOSAN SIA, Latvia, RC No. FSZ 2011/10117);

4. Variable volume dispensers that allow to take liquid volumes from 0.5-10 µl, 10-100 µl or 20-200 µl, 100-1000 µl (e.g., Eppendorf Research Plus, Germany, RC No. FSZ 2011/11028 dated November 15, 2011 or Biohit, Finland, RC No. FSZ 2012/12201 dated May 18, 2012);

5. Refrigerator from 2 to 8°C with freezer below -16 °C (e.g., combined laboratory refrigerator XL-250 POZIS, XL-250-1 POZIS according to TS 9452-203-07503307-2012, manufactured by POSIS JSC, RC No. RZN 2016/4043 dated June 3, 2024);

6. Cycler[3] with real-time fluorescence detection in channels corresponding to FAM/Green, HEX/Yellow, ROX/Orange, Cy5/Red fluorophores:

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

- CFX96 (BioRad, USA, RC No. FSZ 2008/03399 dated June 21, 2016),

- DTprime (NPO DNA-Technology LLC, Russia, RC No. FSR 2011/10229 dated March 3, 2011),

- QuantStudio 5 (Thermo Fisher Scientific, USA, RC No. RZN 2019/8446 dated June 6, 2019),

- FLUORITE (Xian TianLong Science and Technology Co, China, RC No. RZN 2022/16415 dated April 4, 2024).

For configuration forms 1-2, it is possible to conduct the analysis using Rotor-Gene-Q detecting cycler, Qiagen, Germany (RC No. FSZ 2010/07595 dated August 10, 2010).

ATTENTION! Configuration forms 3 and 4 must be used in conjunction with plate-type devices.

Required materials and reagents not included in the kit:

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

1. Disposable tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA); when using an aspirator with a flask trap, disposable tips up to 1000 µl without an aerosol barrier (e.g., Axygen, USA);
2. 1.5 ml disposable Eppendorf tubes;
3. Thin-walled disposable PCR tubes with an optically transparent cap:
   - 0.1 or 0.2 ml PCR tubes,
   - 0.2 ml PCR tubes in strips,
   - PCR plates with optically transparent film (e.g., Axygen, USA);
4. Lab coat and talc-free disposable gloves;
5. Container with disinfectant solution;
6. Test tube racks 0.1-0.2 ml tubes or for 0.2 ml tubes in strips (e.g., InterLabService, Russia);
7. For DNA isolation, a rack for 1.5 ml tubes (e.g., InterLabService, Russia).
8. Magnetic rack for 1.5 ml Eppendorf type tubes;
9. For configuration forms 1 and 3, DNA isolation kit (see Section 8.2 of the Instructions)

Additional materials not included in the product

1. 2200 µl 96DW deep-well plates, extraneous DNA/RNA and DNase/RNase free (RC No. FSZ 2009/05562 dated March 16, 2022);
2. 96 tip comb for deep-well magnets for 96DW plate format (RC No. FSZ 2009/05562 dated March 16, 2022);
3. 100 ml solution reservoirs (e.g., Biologix, China).

8. Test samples

Test sample type

Testing material is DNA samples, isolated from clinical material: rash scrapings, vesicular fluid, cerebrospinal fluid, conjunctival discharge, peripheral blood plasma, cord blood plasma, capillary blood, amniotic fluid, saliva, oropharyngeal washes, oropharyngeal swabs.

Collect peripheral blood and cord blood plasma in a tube with 6% EDTA or 6% EDTA and gel.

Collect capillary blood in a tube with 6% EDTA.

8.1. Clinical material collection

ATTENTION! Before starting work, review methodological recommendations "Collection, transportation, storage of biological material for PCR diagnostics: methodological recommendations", developed by the Central Research Institute of Rospotrebnadzor.[4]

Clinical material collection and its packaging is carried out by an employee of a medical organization trained in the requirements and rules of biological safety when working and collecting material suspected of infection with microorganisms of pathogenicity group III.

8.1.1. Rash scrapings

Collect PCR material using a swab into disposable polypropylene screw-on or tightly closed 1.5 or 2.0 ml tubes (for example, microcentrifuge tubes, Axygen, Inc., USA or similar) with a transport medium (sterile saline solution, 0.01 M potassium phosphate buffer, pH 7.0).

[4] Taking, transportation, and storage of biological material for PCR diagnostics: methodological recommendations / Domonova E.A., Tvorogova M.G., Podkolzin A.T. [et al.]. Moscow: FBIS Central Research Institute of Epidemiology, 2021.

Pre-treat the affected skin area with a swab soaked in a 70% ethyl alcohol solution. Transfer the test material into a tube using forceps. Close the tube tightly with a cap. The surface crusts and scales in the center of the ring-shaped rashes are not suitable for testing!

Storage and transportation:

- at 18°C... 25°C – up to 1 month;
- at -68°C and below – for a long time.

It is allowed to freeze and thaw the material once.

8.1.2. Vesicular fluid

Collect PCR material in polypropylene screw-on or tightly closed 1.5-2.0 ml tubes (for example, microcentrifuge tubes, Axygen, Inc., USA or similar) with a transport medium.

Before sampling treat skin with a swab soaked in a 70% ethyl alcohol solution. Remove crusts or cover of the vesicles from the skin with a scalpel and forceps, then make a puncture at the base with a sterile needle, tilting its free end down to facilitate the collection of contents into a tube with 0.5 ml of transport medium. To speed up sample collection, press additionally on top of the skin with forceps.

Storage and transportation:

- at 18°C... 25°C – up to 48 hours;
- at 2°C… 8°C – up to 7 days;
- at -20°C and below – for a long time.

It is allowed to freeze and thaw the material once.

8.1.3. Cerebrospinal fluid

Collect PCR material into 5.0 ml screw cap tubes (e.g., Axygen, Inc. (USA) or similar) or disposable polypropylene screw-on or tightly closed 2.0 ml tubes (e.g., microcentrifuge tubes, Axygen, Inc., USA or similar) or a plastic 30.0 ml container for biological samples collection, storage and transportation for testing (sterile, individually packaged) (e.g., Combitek Plastic LLC, Russia or similar).

Collect at least 1.0 ml of cerebrospinal fluid by aspiration into a tube or container by puncturing the lumbar, suboccipital region or cerebral ventricles with puncture needles. Close the tube or container tightly with a cap.

Storage and transportation:
- at 2°C… 8°C – up to 24 hours;
- at -24°C... -16°C – up to 3 months;
- at -68°C and below – for a long time.

It is allowed to freeze and thaw the material once.

8.1.4. Conjunctival discharge

Collect PCR material using a swab into disposable polypropylene screw-on or tightly closed 1.5 or 2.0 ml tubes (e.g., microcentrifuge tubes, Axygen, Inc., USA or similar) with a transport medium.

Collect material under local anesthesia (e.g., 2 drops of Dicaine (0.3% solution)). Pulling back the lower eyelid, rotate the applied part of the swab along the conjunctiva 4-5 times, including the inner and outer eye corners. Transfer the swab into a tube with 0.5 ml of transport medium. Brake off the applied part of the swab containing the test material and leave it in a tube with transport medium. Close the tube tightly with the cap, avoiding gaps and crumpling of the inside of the cap. If it is impossible to break off the applied part of the swab, immerse it in the transport medium and, pressing it against the inside of the tube, rotate for 5-10 seconds, after which remove the swab and close the tube tightly. It is not allowed to use scissors to cut off the applied part of the swab!

Storage and transportation:
- at 18°C... 25°C – up to 6 hours;
- at 2°C… 8°C – up to 3 days;
- at -24°C... -16°C – up to 7 days;
- at -68°C and below – for a long time.

8.1.5. Peripheral blood plasma and cord blood plasma.

Collect PCR test material with a disposable needle (0.8–1.1 mm diameter) into a tube with an anticoagulant.

Collect blood in a tube with 6% EDTA or 6% EDTA and gel.

Do not use heparin as an anticoagulant!

Right after blood collection, gently turn the closed tube with blood upside down several times so that the blood in the tube is thoroughly mixed with the anticoagulant. After gentle mixing, place the tube in a rack.

To obtain blood plasma, centrifuge whole blood tubes at 600 g (e.g., 3000 rpm for a centrifuge MiniSpin, Eppendorf, Germany) for 10 minutes at a 18°C... 25°C. Next, transfer at least 1.0 ml of blood plasma aliquot to 2.0 or 5.0 ml tubes using a filter tip. Transfer blood plasma into a new tube within 6 hours after the blood sample collection.

Storage and transportation:

- at 2°C… 8°C – up to 5 days;
- at -24°C... -16°C – up to 3 months;
- at -68°C and below – for a long time.

It is allowed to freeze and thaw the material once.

8.1.6. Capillary blood

Collect PCR material with a disposable needle (0.8–1.1 mm diameter) or a blood lancet into a tube with an anticoagulant.

Collect blood in a tube with 6% EDTA.

Do not use heparin as an anticoagulant!

Right after blood collection, gently turn the closed tube with blood upside down several times so that the blood in the tube is thoroughly mixed with the anticoagulant. After gentle mixing, place the tube in a rack.

It is not allowed to freeze whole blood samples before DNA isolation!

Whole blood samples storage and transportation:

- at 18°C... 25°C – up to 2 hours;
- at 2°C… 8°C – up to 3 days from the moment of the material collection.

It is not permitted to freeze whole blood samples!

8.1.7. Amniotic fluid

Collect PCR material in disposable polypropylene screw-on or tightly closed 2.0 ml tubes (e.g., microcentrifuge tubes, Axygen, Inc., USA or similar) or 5.0; 10.0 ml screw cap tubes (e.g., Axygen, Inc., USA or similar).

Collect at least 1.0–2.0 ml of the amniotic fluid by aspiration into a tube during the amniocentesis procedure. Close the tube tightly with a cap.

Storage and transportation:
- at 2°C… 8°C – up to 24 hours;
- at -24°C... -16°C – up to 1 month;
- at -68°C and below – for a long time.
It is allowed to freeze and thaw the material once.

8.1.8. Saliva

Collect PCR test material in disposable polypropylene screw-on or tightly closed 2.0 ml tubes (e.g., microcentrifuge tubes Axygen, Inc., USA or similar) or 5.0 ml screw cap tubes (e.g., Axygen, Inc., USA or similar) or a plastic 30.0 ml container for biological samples collection, storage and transportation for testing (sterile, individually packaged) (e.g., Combitek Plastic LLC, Russia or similar).

Rinse the mouth three times with 0.9% sodium chloride solution or boiled water. Collect at least 1.0–2.0 ml of saliva in a tube or container, close tightly a cap.

Storage and transportation:
- at 18°C... 25°C – up to 6 hours;
- at 2°C… 8°C – up to 24 hours;
- at -24°C... -16°C – up to 7 days;
- at -68°C and below – for a long time.
It is allowed to freeze and thaw the material once.

8.1.9. Oropharyngeal washes

Collect PCR material into a plastic 60.0 ml container for biological samples collection, storage and transportation for testing (sterile, individually packaged) (e.g., Combitek Plastic LLC, Russia or similar).

First rinse mouth once with 0.9% sodium chloride solution or boiled water. After that, rinse thoroughly the oropharynx with 25.0–40.0 ml of 0.9% sodium chloride solution for 10-15 seconds. Collect the wash liquid in a container and close the cap tightly.

Storage and transportation:
- at 18°C... 25°C – up to 6 hours;
- at 2°C… 8°C – up to 3 days;
- at -24°C... -16°C – up to 7 days;
- at -68°C and below – for a long time.
It is allowed to freeze and thaw the material once.

8.1.10. Oropharyngeal swabs

Collect PCR material using a swab into disposable polypropylene screw-on or tightly closed 1.5-2.0 ml tubes (for example, microcentrifuge tubes, Axygen, Inc., USA or similar) with a transport medium.

Make rotational movements with the applied part of the swab along the surface of the tonsils, palatine arches and the posterior wall of the oropharynx. Transfer the swab into a tube with 0.5 ml of transport medium (as a transport medium, it is recommended to use a reagent for clinical material collection, transportation and DNA isolation DNA-Fast, according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia registration certificate No. RZN 2021/15428 dated June 5, 2023). Brake off the applied part of the swab containing the test material and leave in a tube with transport medium. Close the tube tightly with a cap, avoiding gaps and crumpling of the inside of the cap. If it is impossible to break off the applied part of the swab, immerse it in the transport medium and, pressing against the inside of the tube, rotate for 5-10 seconds, after which remove the swab and close the tube tightly. It is not allowed to use scissors to cut off the applied part of the swab!

Storage and transportation:

- at 18°C... 25°C – up to 6 hours;
- at 2°C… 8°C – up to 3 days;
- at -24°C... -16°C – up to 7 days;
- at -68°C and below – for a long time.

It is allowed to freeze and thaw the material once.

ATTENTION! Avoid repeated sample freezing and thawing.

Accounting, storage, transfer and transportation of clinical material suspected of herpesvirus infection presence should be carried out in accordance with current sanitary and epidemiological rules on the work safety with microorganisms of pathogenicity groups III–IV (SP 1.3.3118-13) and current sanitary rules on accounting, storage, transfer and transportation of microorganisms of pathogenicity groups I– IV.

Clinical material (Class B) disposal, as extremely epidemiologically hazardous waste, is carried out in accordance with SanPiN 2.1.3684-21.

8.2. DNA sample collection

It is recommended to use the following DNA isolation reagent kits when using configuration forms 1 and 3:

- Reagent for clinical material collection, transportation and DNA isolation DNA-Fast according to TS 21.20.23-016-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/14885 dated June 5, 2023).

- Reagent kit for DNA/RNA isolation from the clinical material NA-Extra according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated June 5, 2023).

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

DNA test samples storage conditions

- at 2°C... 8°C – up to 24 hours;
- at -22°C... -18 °C – up to 1 month;
- below -70°C – for a long time.

9. Components preparation for testing

Installation, assembling, adjustment, calibration of a medical device for commissioning is not required.

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

For configuration forms 1 and 2

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

Before the reaction mixtures preparation, wet clean the PCR box, as well as the equipment and materials contained in it, using disinfectants suitable for use in PCR laboratories, and turn on the UV lamp for 20 -30 minutes. Before testing thaw the kit components at room temperature.

1. Before the sample preparation stage, mix thoroughly the ICS tube content (if the kit was stored at subzero temperatures, thaw completely the tube contents at room temperature).

2. Mix thoroughly the tubes, containing DNA isolated for testing, PCR Buffer, Primer Mix, NC and PC, turning each tube upside down 10 times or mixing using a vortex at low speed for 3-5 seconds, then remove drops from the tube caps by short centrifugation.

3. Select the required number of 0.1– 0.2 ml PCR tubes based on the calculation: number of test samples + 1 (PC) + 1 (NC).

For configuration forms 3 and 4

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

Before the lyophilized kit components reconstitution, wet clean the PCR box, as well as the equipment and materials located in it, using disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20–30 minutes. Before testing, thaw the kit components at room temperature.

Before starting work, keep the kit components at 18°C... 25°C for 30 minutes.

Open the ICS bottle. Add 960 µl of ICS reconstitution solution. Close the bottle tightly. Mix gently, keep at 18°C... 25°C for 15 minutes, then mix again. After dilution, store at -22°C... -18°C up to 1 year or at 2°C... 8°C up to 1 month.

Open the PC bottle and add: 704 µl of PC reconstitution solution. Close the bottle tightly. Mix gently, keep at 18°C... 25°C for 15 minutes, then mix again. After dilution, store at -22°C... -18°C up to 1 year or at 2°C... 8°C up to 1 month.

10. Testing procedure

Only specially trained personnel with PCR analysis skills are allowed to work with the kit!

A. DNA isolation

DNA isolation using a reagent kit for DNA isolation DNA-extra-VZV (VZV-test, configuration forms 2 and 4) can be performed for the following biomaterial types: rash scrapings, vesicular fluid, cerebrospinal fluid, conjunctival discharge, peripheral blood plasma, umbilical cord blood plasma, capillary blood, amniotic fluid, saliva, oropharyngeal washes, oropharyngeal swabs.

Layering or precipitation does not affect the solutions quality. If there is sediment or layering in the bottles, heat them at 70°C and mix until the precipitate is completely dissolved and the solutions are homogenized.

Mix all kit components before use.

Manual DNA isolation protocol:

For each test sample, prepare and label one 1.5–2.0 ml tube (PC and NC samples do not undergo the DNA isolation procedure).

1. Add into each tube:

Binding Buffer – 500 µl;

Magnetic Bead Solution – 10 µl;

ICS – 10 µl.

To isolate a large number of samples, the mixture can be pre-prepared in a separate tube according to the following scheme:

Binding buffer – 500***(n+1)** µl;

Magnetic Bead Solution – 10***(n+1)** µl;

ICS – 10***(n+1)** µl;

where **n** – number of samples to be isolated

Transfer 520 µl of the Binding Buffer, magnetic beads and ICS mixture into each tube.

2. Add 100 µl of a clinical material sample into each tube, mix using a vortex for 3-5 seconds. For lysis, incubate the tubes at 70°C for 5

minutes, mixing the solution 1-2 times using a vortex during incubation.

3. Upon lysis completion, transfer the tubes to a test tube rack and incubate the DNA binding mixture at room temperature for 10 minutes, mixing the solution 2-3 times during incubation by turning the tubes over.

4. Place the tubes in a magnetic separation rack, wait until the beads collect completely on the tube wall (usually it takes 1-2 minutes) and remove the supernatant using a dispenser or aspirator.

5. Add 700 µl of Wash Solution No. 1 into the tubes, close the caps tightly, resuspend the magnetic beads using a vortex, and remove the drops by short centrifugation.

6. Place the tubes in a magnetic separation rack, wait until the beads collect completely on the tube wall and remove the supernatant.

7. Add 700 µl of Wash Solution No. 2 into the tubes, close the caps tightly, resuspend the magnetic beads using a vortex, and remove the drops by short centrifugation.

8. Place the tubes in a magnetic separation rack, wait until the beads collect completely on the tube wall and remove the supernatant.

9. Repeat steps 7 and 8.

10. Place the tubes with the caps open in a thermostat and incubate at 70°C for 5 minutes to dry the magnetic beads and remove residual alcohol.

11. Add 50 µl of eluent into the tubes using a separate filter tip. Resuspend carefully magnetic beads by pipetting, and close the caps tightly.

12. Incubate the tubes at 70°C for 10 minutes. During incubation, mix the contents of the tubes 2-3 times shaking gently the sediment.

13. Place the tubes in a magnetic separation rack, wait until the beads collect completely on the tube wall.

14. Transfer the supernatant containing the isolated DNA into new tubes. ATTENTION! The isolated DNA is selected without removing the tubes from the magnetic separation rack.

Automatic DNA isolation protocol:

DNA isolation procedure with a reagent kit for DNA isolation DNA-extra-VZV using pipetting workstations (e.g., Tecan Freedom EVO® series or similar):

1. Transfer the entire contents of the "Magnetic Beads" tube (960 µl) into a "Binding Buffer" bottle. This mixture can be stored for 7 days. Mix thoroughly the prepared suspension of magnetic beads in "Binding Buffer" before each use;
2. Prepare an automated sample preparation workstation in accordance with the instructions for its use;
3. Pour the prepared solutions from the bottles into the cuvettes and load them to the workstation in the order described in the workstation operation protocol.
4. Load special tips, tubes for performing DNA isolation reactions, and tubes for isolated DNA into the station in the order described in the station operation protocol.
5. Upload the corresponding isolation protocol for working with DNA-extra-VZV reagent kit in the workstation software (the protocol file is available upon request).
6. Start the isolation protocol.
7. Upon DNA isolation completion, remove the tubes with the isolated DNA from the workstation.
8. Remove used consumables from the workstation and clean according to the instructions.

DNA isolation procedure with a reagent kit for DNA isolation DNA-extra-VZV using magnetic stirrer stations (KingFisher Flex 96 or similar)

Before isolation, add reagents to 96 deep-well plates according to the following scheme:

Table 18 – Reagent application scheme for automatic isolation with magnetic stirrer stations

Plate number	Component	Component amount per 1 plate well, µl
Plate 1	Binding Buffer	500
	Magnetic Beads	10
	ICS	10
Plate 2	Wash solution No. 1	700
Plate 3	Wash solution No. 2	700
Plate 4	Wash solution No. 2	700
Plate 5	Eluent	100
Plate 6	Tip comb	-

1. Transfer the entire contents of the "Magnetic Beads" tube (960 µl) into a "Binding Buffer" bottle. This mixture can be stored for 7 days. Mix thoroughly the prepared suspension of magnetic beads in "Binding Buffer" before each use.
2. Label 96 deep-well plates and add reagents into them according to the scheme (Table 18). A single-channel or multi-channel automatic variable volume dispenser can be used to introduce reagents. Reagents can also be added using an automated sample preparation workstation. Attention: add each reagent with a separate tip, the remains of one reagent must not enter the other.
3. Prepare the equipment according to the instructions for its use.

4. Upload the isolation protocol into the device (the protocol file is available upon request).

5. Add 100 µl of the clinical material tested sample into the wells of plate 1.

6. Arrange the plates and the tip comb for magnetic rods in accordance with the instructions of the device, and start the DNA isolation procedure according to the uploaded protocol of the automated workstation.

7. At the end of the device operation, an eluent containing the isolated DNA is in the wells of the plate 5.

Sample preparation using a reagent kit for DNA/RNA isolation from clinical material NA-Extra (together with configuration forms 1 and 3) can be performed for the following biomaterial types: peripheral blood plasma, cord blood plasma, capillary blood, oropharyngeal swabs.

The stated sensitivity - 500 copies/ml.

Sample preparation is carried out in accordance with the instructions for a reagent kit for DNA / RNA isolation from the clinical material NA-Extra.

Sample preparation using a reagent for clinical material collection, transportation and DNA isolation DNA-Fast (together with configuration forms 1 and 3) can be carried out for the following biomaterial types: saliva, oropharyngeal swabs.

The stated sensitivity - 1000 copies/ml.

Sample preparation is carried out in accordance with the instructions for the reagent for clinical material collection, transportation and DNA isolation DNA-Fast.

B. PCR preparation

(carried out in the pre-PCR area - a room for reagents dispensing and preparing for PCR-amplification)

PCR layout scheme for samples prepared using NA-Extra kit (for configuration form 1) and DNA isolation reagent kit DNA-extra-VZV (configuration form 2)

Total reaction volume – 25 µl.

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

To prepare a reaction mixture for 1 reaction you need:

PCR Buffer – 5 µl;

Primer Mix – 5 µl;

Sample [5] – 15 µl.

The total number of tubes, including PC and NC, is calculated using the formula:

N = n + 2, where n – number of samples

Prepare the reaction tubes in the following order:

1. Label 0.2 ml PCR tubes;
2. Add 5 µl of PCR Buffer into each tube;
3. Add 5 ml of Primer Mix;
4. Add 15 µl of the sample (including PC and NC, without isolation) into the corresponding tubes;
5. Close the tubes;
6. To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a microcentrifuge-vortex.

The samples are ready for amplification.

[5] Hereinafter in this chapter, "Sample" refers to the DNA tested sample, PC or NC

To study a large number of samples, it is possible to prepare a mixture of PCR Buffer and Primer Mix:

1. In a separate tube mix the specified volumes of PCR Buffer and Primer Mix.

The amounts of PCR Buffer and Primer Mix to be added are determined by the following formula:

$V_{PCR\ Buffer} = 5 * (n + 1)$ µl;

$V_{Primer\ Mix} = 5 * (n + 1)$ µl;

where n – the total number of tubes with samples (including NC and PC).

2. Add 10 µl of a mixture of PCR Buffer and Primer Mix into each PCR tube;
3. Add 15 µl of the sample (including PC and NC, without isolation) into the corresponding PCR tubes;
4. Close the tubes, mix the contents;
5. To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a microcentrifuge-vortex.

PCR layout scheme for samples prepared using DNA-Fast reagent (for configuration form 1)

Total reaction volume – 25 µl.

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

To prepare a reaction mixture for 1 reaction you need:

PCR Buffer – 5 µl;

Primer Mix – 5 µl;

ICS – 2.5 µl;

NC – 7.5 µl;

sample – 5 µl.

The total number of tubes, including PC and NC, is calculated using the formula:

N = n + 2, where n – number of samples

Prepare the reaction tubes in the following order:

1. Label 0.2 ml PCR tubes;
2. Add 5 µl of PCR Buffer into each tube;
3. Add 5 µl of Primer Mix into each tube;
4. Add 2.5 µl of ICS into each tube;
5. Add 7.5 µl of NC into each tube;
6. Add 5 µl of the sample (including PC and NC) into the corresponding tubes;
7. Close the tubes;
8. To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a microcentrifuge-vortex.

The samples are ready for amplification.

To study a large number of samples, it is possible to prepare a mixture of PCR Buffer, Primer Mix, ICS and NC:

1. In a separate tube, mix the specified volumes of PCR Buffer, Primer Mix, ICS and NC. The volumes of the added PCR Buffer, Primer Mix, ICS and NC are determined by the formula:

$V_{PCR\ Buffer} = 5 * (n + 1)$ µl;

$V_{Primer\ Mix} = 5 * (n + 1)$ µl;

$V_{ICS} = 2.5 * (n + 1)$ µl;

$V_{NC} = 7.5 * (n + 1)$ µl;

where n – the total number of tubes with samples (including NC and PC).

2. Add 20 µl of a mixture of PCR Buffer, Primer Mix, ICS and NC into each PCR tube;
3. Add 5 µl of the sample (including PC and NC) into the corresponding PCR tubes;
4. Close the tubes, mix the contents;
5. To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a microcentrifuge-vortex.

PCR layout scheme for samples prepared using NA-Extra kit (for configuration form 3) and DNA isolation reagent kit DNA-extra-VZV (configuration form 4)

1. Open the LRM packing, use a stationery knife or scissors to cut off the required number of LRM tubes (including control samples). The tubes should be cut off with a film covering them.
2. Pack unused LRM tubes into a bag with a desiccant, remove air and close tightly. After the first opening store LRS packings at 2°C ... 8°C for up to 3 months.

Total reaction volume – 25 µl (including lyophilizate).

ATTENTION! It is forbidden to change the reaction volume.

3. Label the tubes according to the protocol, including control samples (negative and positive).
4. Carefully remove the film from the tubes, making sure that the lyophilized mixture LRM is not removed along with the film.
5. Add 22 µl of isolated DNA into the corresponding tubes for the tested samples and mix 3-5 times by pipetting, avoiding foam formation. Do not add DNA preparation into the tubes for PC and NC.
6. Add 22 µl of PC into the corresponding tube and mix 3-5 times by pipetting.
7. Add 22 µl of NC (without isolation) into the corresponding tube and mix 3-5 times by pipetting.
8. Seal the plate/tubes with the film included in the kit.
9. To remove drops from the walls, centrifuge the tubes for 30 seconds.
10. Keep the tubes for 3–5 minutes to reconstitute the reaction mixture at room temperature.

PCR layout scheme for samples prepared using DNA-Fast reagent (for configuration form 3)

1. Open the LRM packing, use a stationery knife or scissors to cut off the required number of LRM tubes (including control samples). The tubes should be cut off with a film covering them.
2. Pack unused LRM tubes into a bag with a desiccant, remove air and close tightly. After the first opening store LRS packings at 2°C ... 8°C for up to 3 months.

Total reaction volume – 25 µl (including lyophilizate).

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

3. Label the tubes according to the protocol, including control samples (negative and positive).
4. Carefully remove the film from the tubes, making sure that the lyophilized mixture LRM is not removed along with the film.
5. Add into the corresponding tubes for the tested samples (including for PC):

- 15 µl of NC,
- 2.5 µl of ICS,
- 5 µl of isolated DNA (or PC and NC into the corresponding labeled tubes) and mix 3-5 times by pipetting, avoiding foam formation.

6. Seal the plate/tubes with the film included in the kit.
7. To remove drops from the walls, centrifuge the tubes for 30 seconds.
8. Keep the tubes for 3–5 minutes to reconstitute the reaction mixture at room temperature.

B. DNA PCR amplification with hybridization-fluorescence detection of amplification products in real time

(carried out in the PCR area - a room for PCR amplification)

1. Install the tubes in the reaction module of the real-time PCR device. It is recommended to install the tubes in the center of the thermal block to evenly press the tubes with the heated lid;

2. Program the device to perform the corresponding program of amplification and fluorescent signal detection, following the instructions for the used device. PCR protocol for DTprime cyclers (NPO DNA Technology LLC, Russia), QuantStudio 5 (Thermo Fisher Scientific, USA), Rotor-Gene Q (Qiagen, Germany), CFX 96 (Bio-Rad, USA), FLUORITE (AO Vector-Best, Russia) is specified in Table 19.

Table 19 – PCR protocol for VZV-test

Stage	Temperature, ˚C	Time, min:sec	Detection channels	Total cycles
1	95	02:00	-	1
2	95	00:15	-	5
	64	00:20		
3	95	00:15	-	40
	64	00:20	FAM/Green, HEX/Yellow	

3. Specify the samples number and identifiers, mark the tubes location in the cells of the reaction module according to their layout.

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

Attention! When using configuration forms 3 and 4 for DTprime cycler, it is required to set the exposures for the channels: Fam – 500, Hex – 1000.

5. Start PCR with a fluorescent signal detection.

6. Upon the program completion start analyzing the results.

11. Results registration and interpretation

The results are recorded upon PCR completion with the software of the used device.

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 results are interpreted using Ct values of FAM/Green and HEX/Yellow channels (Table 1).

First, evaluate the reaction and Ct values in the control samples. Begin results interpretation in the tested samples only with PC and NC correct reactions.

Result interpretation in control samples

PCR testing is considered correct if the following results are obtained for negative and positive control samples (Table 20).

Table 20 – The study results for negative and positive control samples corresponding to the correct PCR

Control sample	Selected fluorophore	
	FAM/Green	HEX/Yellow
NC	absent	not considered
PC	Ct ≤ 28	Ct ≤ 28

When obtained values for a negative control sample differ from those indicated in Table 20, the results of the entire series are considered unreliable. In this case, it is required to take special measures to eliminate possible contamination.

When obtained values for a positive control sample differ from those indicated in Table 20, repeat amplification of the entire samples batch. When re-obtaining values for PC that differ from those indicated in Table 20, replace the reagents.

Result interpretation in the tested clinical samples

Result interpretation principles are listed in Table 21.

Table 21 – Result interpretation principle

Ct values		Result
Specificity channel **(FAM/Green)**	ICS channel **(HEX/Yellow)**	
Ct ≤ 35	not considered	Varicella Zoster virus DNA **detected**
Ct > 35 or absent	Ct ≤ 35	Varicella Zoster virus DNA **not detected or below the detection limit**
Ct > 35 or absent	Ct > 35 or absent	**Invalid** result

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

In case of an invalid result, the conclusion is not issued, it is required to re-take the biomaterial from the patient and re-test it.

Diagnostic value of the obtained study result:

The obtained results can be used to diagnose diseases caused by Varicella Zoster virus.

A specialist doctor should determine a diagnosis and prescribe treatment based on clinical observations, medical history and epidemiological information in combination.

12. Reagent kit storage, transportation and operation conditions

Storage

Table 22 – Storage conditions

Configuration form	Reagent kit	Storage conditions	Duration	Note
CF 1 - VZV-test	VZV-test	at -22°C… -18°C	12 months	It is allowed to freeze/thaw up to 10 times
		at 2°C... 8°C	up to 90 days	
CF 2 VZV-test-extra	DNA-extra-VZV	at 2°C... 30°C	12 months	Do not freeze!
	VZV-test	at -22°C… -18°C	12 months	It is allowed to freeze/thaw up to 10 times
		at 2°C... 8°C	up to 90 days	
CF 3 VZV-test-Lyo	VZV-test-Lyo	at 2°C... 25°C	12 months	Do not freeze!
CF 4 VZV-test-extra-Lyo	DNA-extra-VZV	at 2°C... 30°C	12 months	Do not freeze!
	VZV-test-Lyo	at 2°C... 25°C	12 months	Do not freeze non-reconstituted lyophilized mixture!

Reagent kits stored in violation of the regulated regime cannot be used.

Transportation

Table 23 – Transportation conditions

Configuration form	Reagent kit	Storage conditions	Duration	Note
CF 1 - VZV-test	VZV-test	at -22°C… -18°C	12 months	It is prohibited to transport at temperatures above 25°C!
		at 2°C... 8°C	up to 90 days	
		at 8°C... 25°C	up to 5 days	
CF 2 VZV-test-extra	DNA-extra-VZV	at 2°C... 30°C	12 months	Do not freeze!
	VZV-test	at -22°C… -18°C	12 months	It is prohibited to transport at temperatures above 25°C!
		at 2°C... 8°C	up to 90 days	
		at 8°C... 25°C	up to 5 days	
CF 3 VZV-test-Lyo	VZV-test-Lyo	at 2°C... 25°C	12 months	Do not shake!
CF 4 VZV-test-extra-Lyo	DNA-extra-VZV	at 2°C... 30°C	12 months	Do not freeze!
	VZV-test-Lyo	at 2°C... 25°C	12 months	Do not shake!

Transport VZV-test reagent kit of all configuration forms in all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

To ensure compliance with transportation conditions throughout the entire transportation period, place a reagent kit, configuration forms 1 (VZV-test) and 2 (VZV-test-extra) 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 the thermal container with the transported reagent kits, as well as the thermal container volume are selected depending on the transportation duration and conditions.

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

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

Shelf life

The VZV-test reagent kit shelf life in all configuration forms – 12 months from the acceptance date of the manufacturer's QCD, if compliant with all transportation, storage and operation conditions.

A reagent kit cannot be used after the expiration date.

Shelf life of opened kit components

Table 24 – Storage conditions and shelf life of opened kit components

Configuration form	Reagent kit	Storage conditions	Shelf life
CF 1 - VZV-test	VZV-test	at -22°C... -18°C	12 months
CF 2 VZV-test-extra	DNA-extra-VZV	at 2°C... 30°C	12 months
	VZV-test	at -22°C... -18°C	12 months
CF 3 VZV-test-Lyo	VZV-test-Lyo	PC and ICS after reconstitution at -22°C... -18°C	12 months
		PC and ICS after reconstitution at 2°C to 8°C	1 month
		after opening the LRM packaging (a ziplock bag) at 2°C... 8°C	3 months
CF 4 VZV-test-extra-Lyo	DNA-extra-VZV	at 2°C... 30°C	12 months
	VZV-test-Lyo	PC and ICS after reconstitution at -22°C... -18°C	12 months
		PC and ICS after reconstitution at 2°C to 8°C	1 month
		after opening the LRM packaging (a ziplock bag) at 2°C... 8°C	3 months

13. Disposal

Reagent kits that have become unusable, including due to expiration dates, must be disposed of in accordance with the requirements of SanPiN 2.1.3684-21.

According to the medical waste classification, 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 disposable labelled 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 MU 287-113 (Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices).

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.

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

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

14. Warranty, contacts

The manufacturer guarantees VZV-test reagent kit quality and safety during shelf life if compliant with transportation and storage requirements as well as rules of operation.

If you have any complaints about the kit quality, undesirable events or incidents, please contact:

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

	Contains sufficient for < n > tests
	Refer to the instructions for use
	In vitro diagnostic medical device
	Temperature limitation
	Batch code or Lot number
	Use by…
	Manufacture date
	Fragile, handle with care
	This side up. Do not turn over or tip on its side. It must be stored and transported upright only.

Annex B

Designation	Document name
GOST ISO 14971-2021	Medical devices. Application of risk management to medical devices.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, reagent kits, the test systems, control materials, culture medium. Requirements to devices and supporting documentation.
GOST R ISO 23640-2015	In vitro diagnostic medical devices. Stability evaluation of in vitro diagnostic reagents.
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 15223-1-2023	Medical devices. Symbols to be used with information to be supplied by the manufacturer. Part 1. General requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.
GOST R ISO 17511-2022	In vitro diagnostic medical devices. Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human biological samples.