DNA-TECHNOLOGY

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CE IVD

For professional use only

Varicella zoster virus REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

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R1-P206-23/4EU

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1. INTENDED USE

The **Varicella zoster virus REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Varicella zoster virus REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Varicella zoster virus REAL-TIME PCR Detection Kit** is designed to detect Varicella zoster virus nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: blood, vesicular fluid, epithelial cell swabs from erosive and ulcerative elements, cerebrospinal fluid, conjunctival discharge.

Indications for the use: symptoms of chickenpox and shingles, monitoring the effectiveness of antiviral treatment, differential diagnosis of infections with similar clinical implications.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Varicella zoster virus REAL-TIME PCR Detection Kit.**

The **Varicella zoster virus REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **Varicella zoster virus REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the Varicella zoster virus product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Varicella zoster virus	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Varicella zoster virus REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **Varicella zoster virus REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **Varicella zoster virus REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **Varicella zoster virus REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P206-S3/4EU and R1-P206-23/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	960 µL (20 µL in each tube)	48 tubes or 6 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps*	6 8-caps		

*- for detection kit packaged in strips REF R1-P206-S3/4EU

All components are ready to use and do not require additional preparation for operation.

The **Varicella zoster virus REAL-TIME PCR Detection Kit** is intended for single use and designed for 48 tests (46 defined samples, one positive control and one negative control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single-use sterile flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;

- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) and **PREP-MB RAPID** (REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips (in case of using package S, strips REF R1-P206-S3/4EU);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **Varicella zoster virus REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **Varicella zoster virus REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Varicella zoster virus REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Varicella zoster virus REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **Varicella zoster virus REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, vesicular fluid, epithelial cell swabs from erosive and ulcerative elements, cerebrospinal fluid, conjunctival discharge, depending on professional prescription.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

General requirements

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results. PCR research is a direct method, so taking of biological material must be carried out from the location of the infectious process.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are the presence of hemoglobin in the DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL DNA sample.

To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible

The features of the blood sampling:

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

The features of the cerebrospinal fluid sampling:

Cerebrospinal fluid (about 500 µL) is collected in a sterile container, closed tightly and marked.

Sample preparation (cerebrospinal fluid)

It is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA, PREP-MB RAPID** kits:

1. Transfer 500 µL of the material into a 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperatures (from 18 °C to 25 °C).
3. Remove the supernatant, leaving 50 µL in tube (precipitate + liquid fraction).
4. Add 500 µL of a sterile saline solution to the precipitate.
5. Vortex the tube for 3-5 seconds, then spin for 3-5 seconds.
6. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
7. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS**. Tightly close the tubes.

The resulting material is ready for DNA extraction.

The features of the vesicular fluid, swabs from erosive and ulcerative elements sampling:

The material is collected using special sterile disposable instruments - probes or tampons, according to the established procedure. After taking the clinical material, transfer the probe to a test tube with a transport medium intended by the manufacturer for transporting and storing samples of biological material for PCR studies, and thoroughly rinse it for 10-15 seconds, avoiding liquid splashing. Then remove the probe from the solution by pressing it against the tube wall, squeeze out the excess liquid, remove the probe and dispose it. Close the test tube tightly and mark it.

The limitation of the method is the local use of medicines less than 24 hours before the study.

Sample preparation (vesicular fluid, swabs from erosive and ulcerative elements)

When taking swabs into tubes with the **PREP-RAPID** reagent, sample preparation is not required, the material is ready for DNA extracting.

It is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA, PREP-MB RAPID** kits:

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperatures (from 18 °C to 25 °C).
2. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS**. Tightly close the tubes.

The resulting material is ready for DNA extraction.

The features of the conjunctival discharge sampling:

The material is collected using special sterile disposable instruments - tampons, according to the established procedure. If the abundant purulent discharge is presence, it is removed with a sterile cotton tampon moistened with saline. Sampling is taken from the inner surface of the lower eyelid by the movement to the inner corner of the eye slit. It is necessary to hold the eyelid with hands so that the eyelashes do not touch the tampon. After taking the clinical material, transfer the tampon to a test tube with a transport medium intended by the manufacturer for transporting and storing samples of biological material for PCR studies, and thoroughly rinse it for 10-15 seconds, avoiding liquid splashing. Then remove the probe from the solution by pressing it against the tube wall, squeeze out the excess liquid, remove the probe and dispose it. Close the test tube tightly and mark it.

The limitation of the method is the local use of medicines less than 24 hours before the study.

Transportation and storage of the samples

Samples may be transported and stored in physiological saline at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

In case of usage transport media biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID**, **PREP-NA**, **PREP-GS** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-NA, PREP-GS, PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control in volumes as indicated.

Assay procedure for package S

The reagents and tubes should be kept away from direct sun light.

When using package S (R1-P206-S3/4EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1 Mark tubes with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

8.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene Q thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, “C+” and “C-“ for 3-5 seconds and spin down drops for 1-3 seconds.

In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-“ tubes. Avoid paraffin layer break.

8.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, spin is not required).

8.9 Set the tubes/strips into the Real-time Thermal Cycler.

8.10 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (8.9) and run PCR. See tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

Table 3. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]			Holding		Holding
[1] – holding at 25°C is allowed						

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **Varicella zoster virus REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the Varicella zoster virus genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance. To reveal possible contamination a negative control is required.

A negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

For **Varicella zoster virus REAL-TIME PCR Detection Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absence and for internal control is present.

For **Varicella zoster virus REAL-TIME PCR Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing Varicella zoster virus DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of Varicella zoster virus DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **Varicella zoster virus REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with Varicella zoster virus DNA are to be registered positive for specific product (a fragment of the Varicella zoster virus genome). The samples free of Varicella zoster virus DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of Cytomegalovirus, Herpes symplex virus 1,2, Human herpesvirus 6, Human herpesvirus 8, Epstein-Barr virus, HPV 6, HPV 11, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. In a determination of analytical sensitivity, the **Varicella zoster virus REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of VZV DNA per amplification tube. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS). 94 tests were made for each concentration.

The concentration of LCS, copies per amplification tube	Number of repetitions	Number of positive results	% of positive results
5.0	94	94	98.9
2.0	94	89	94.6
0.5	94	60	63.8
0.0	94	0	0.0

Sensitivity of VZV DNA in the sample depends on the sampling and the final volume of the extracted DNA (elution volume).

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of VZV in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID** (at elution in 300 µL)	**PREP-RAPID**
- epithelial cell swabs from erosive and ulcerative elements, conjunctival discharge, vesicular fluid in 500 µL transport medium; - cerebrospinal fluid (extracting from 500 µL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID** REF P-001/1EU, **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EUextraction kits.

12. TROUBLESHOOTING

Table 8. Troubleshooting

	Result	**Possible cause**	**Solution**
C+	-	Operation error PCR inhibition Violation of storage and handlingrequirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issueswith the kit:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **Varicella zoster virus REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
NON STERILE	Non-sterile		Do not reuse
EC REP	Authorized representative in the European Community		Caution