DNA-TECHNOLOGY

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For research use only

HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P213-S3/9ER
R1-P213-23/9ER
R1-P213-UA/9ER

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1. INTENDED USE

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** is designed for detection of *Herpes simplex virus 1* (HSV1) and *Herpes simplex virus 2* (HSV2) DNA in human biological material (urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit.**

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in research methods and rules of work in the laboratory.

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

2. METHOD

Method: polymerase chain reaction (PCR) with detection of the results in real time; qualitative multiplex analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by DNA-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a “hot” start is provided. For package S, “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. “Hot” start for package U is provided by using polymerase whose activity is blocked by antibodies, the activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific binding of primers to the DNA target during the initial heating of the test tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the PCR mix. When a specific product is formed, the DNA probe is destroyed and the effect of the quencher on the fluorescent label stops, which leads to an increase in the fluorescence level recorded by detecting thermal cycler. The number of destroyed probes (and therefore the fluorescence level) increases in proportion to the number of specific amplicons produced. The fluorescence level is measured at each amplification cycle in real time.

The PCR mix includes the internal control (IC), which is intended to assess the quality of the polymerase chain reaction.

The DNA probe used to detect the *Herpes simplex virus 2* (HSV2) amplification product DNA includes the fluorescent dye Fam. The DNA probe used to detect *Herpes simplex virus 1* (HSV1) amplification product DNA includes the fluorescent dye Cy5. The DNA probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex.

Using several fluorescent dyes allows to reduce the number of tubes and biomaterial required for the test, because there is a possibility to register results of different amplification reactions in one tube simultaneously.

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
Herpes simplex virus 2 (**HSV2**)	IC	-	*Herpes simplex virus 1* (**HSV1**)	-

3. CONTENT

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P213-S3/9ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	1,920 µL (20 µL in each)	tubes, 12 strips of 8
Taq polymerase solution	Colorless transparent liquid	1,000 µL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps	12 strips of 8		

Table 3. The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P213-23/9ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	1,920 µL (20 µL in each)	96 individual tubes
Taq polymerase solution	Colorless transparent liquid	1,000 µL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

Table 4. The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** content, package U, tubes for R1-P213-UA/9ER

Reagent	Description	Total volume	Amount
PCR mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR buffer	Colorless transparent liquid	600 µL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

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

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** (package S) is intended for single use and designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** (package U) is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

[1] - marking as C+ is allowed

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following equipment, reagents and consumables are required:

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips[1]	tubes	manual	automated
UV PCR cabinet	yes	yes	yes	yes
Real-time detecting thermal cycler[2]	yes	yes	yes	yes[3]
Vortex mixer[4]	yes	yes	yes	yes
Vortex rotor for 0.2 mL strips	yes	no	no	no
Refrigerator with freezing chamber	yes	yes	yes	yes
Tube rack for 1.5 mL tubes	yes	yes	yes	yes
Tube rack for 0.2 mL tubes	no	yes	yes[5]	no
Tube rack for 0.2 mL strip tubes	yes	no	no	no
Single channel pipettes (dispensers covering 0.5-10; 2.0-20; 20-200; 200-1,000 µL volume range)	yes	yes	yes	yes
RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL)	yes	yes	yes	yes
Pipette rack	yes	yes	yes	yes
RNase and DNase free 1.5 mL microfuge tubes with caps	no	no	yes	yes
RNase and DNase free 0.2 mL PCR tubes or 96-well microplate[6]	no	no	yes	no
Powder-free surgical gloves	yes	yes	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes	yes	yes
DTstream dosing instrument, version 12M1 or 15M1	yes[7]	no	no	yes
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream, or similar	yes[7]	no	no	yes
DTpackplate sealing device	no	no	yes[8]	yes
Centrifuge for PCR microplates (RCF(g) at least 100)	no	no	yes[8]	yes
Polymer thermal film for microplate sealing	no	no	yes[8]	yes
384-well PCR microplate	no	no	no	yes
Transport medium (if necessary), the following are recommended: – **STOR-F** transport medium for biomaterial samples; – **STOR-M**[9] transport medium with mucolytic				
Physiological saline solution 0.9% NaCl (sterile)				
NA extraction reagent kits[10], the following are recommended: – **PREP-NA;** – **PREP-NA PLUS**; – **PREP-GS;** – **PREP-GS PLUS;** – **PREP-RAPID**; – **PREP-OPTIMA**; – **PREP-OPTIMA MAX;** – **PREP-MB-RAPID II;** – **PREP-MB MAX.**				

Notes:
[1] – not used for Rotor-Gene Q detecting thermal cyclers
[2] – hereinafter – detecting thermal cycler; the required parameters are indicated below
[3] – validated with DTprime *X and DTprime II *X detecting thermal cyclers
[4] - DTspin laboratory shaker (DNA-Technology, Russia) is recommended; rotor for 0.2 mL strip tubes is included in the package
[5] – only if using tubes
[6] – not used for DTlite and Rotor-Gene Q detecting thermal cyclers
[7] – in case of automated dosing
[8] – only if using PCR microplates
[9] – not recommended if using **PREP-MB-RAPID II** extraction kit
[10] – the choice of DNA extraction kit is determined by biomaterial type

The following detecting thermal cyclers are validated for work with the **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit**:

- DTprime in DTprime *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime;
- DTprime II in DTprime II *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II;
- DTprime II in DTprime II *X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II *X* (only for package U, automated dosing);
- DTprime in DTprime *X* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime *X* (only for package U, automated dosing);
- DTlite in DTlite *S* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTlite (only for package S and package U, manual dosing, tubes);
- Rotor-Gene Q (manufactured by QIAGEN GmbH, Germany), hereinafter – Rotor-Gene Q (only for package S, tubes);
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96;
- Applied Biosystems QuantStudio 5 (manufactured by Life Technologies Holdings Pte. Ltd., Singapore), hereinafter – Applied Biosystems QuantStudio 5.

For the use of detecting thermal cyclers other than those listed above, please consult the reagent kit manufacturer for consultation.

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.

5.1. Storage conditions

5.1.1. **Package S**

- All components of the **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.
- Paraffin-sealed PCR mix must be stored out of light over the storage period.

5.1.2. **Package U**

- All components of the **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit,** except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.
- PCR mix must be stored out of light over the storage period.
- TechnoTaq MAX polymerase (package U) must be stored in a freezer at the temperatures from minus 22 °C to minus 18 °C over the storage period.

WARNING! The excessive temperature and light can be detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

5.2.1. **Package S**

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2 °C to 25 °C for no longer than 5 days.

5.2.2. **Package U**

- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase (package U) in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes up to 25 °C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. **Package S**

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period;
- Paraffin-sealed PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C and out of light over the storage period;

5.3.2. **Package U**

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period;
- PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C and out of light over the storage period;
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 22 °C to minus 18 °C over the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** must not be used.

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

Conformity of **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or unreliable results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.
- Avoid any direct contact with the biological samples, reagents and materials used to carry out the test. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- 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 only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips. The tips employed must be sterile, free from DNases and RNases and 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 to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as potentially infectious[2, 3]. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 minutes or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must 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 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. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.

[2] - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

[3] - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 minutes before and after the assay. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

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

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

Do not use the kit:

- If the transportation and storage conditions have been violated;
- If the appearance of the reagents does not correspond to the product documentation;
- If the packaging of the kit components is breached;
- After the expiry date.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

7. SAMPLES

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes and epithelial scrapes from oropharynx.

7.1. Interfering substances

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

The following substances are considered to be PCR inhibitors that may be present in the DNA sample: Hemoglobin and pharmaceuticals present in the DNA sample as a result of incomplete removal during DNA extraction from the biomaterial sample, and isopropyl alcohol and methyl acetate remaining in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control and internal control are: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL of the DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

To assess the possible interference of drugs, we selected those potentially present in residual amounts in human biological samples taken from the corresponding biotopes under test (Miramistin®, chlorhexidine bigluconate).

For all the drugs under test no effect was found in concentration up to 10% in biomaterial sample.

7.2. General requirements

7.2.1 PCR analysis refers to direct methods of laboratory research; therefore, the collection of biological material must be carried out from the site of infection localization. The decision to examine the localization is taken by a physician based on the collected anamnesis and the clinical picture of the disease.

7.2.2 The quality of sampling, sample storage, transport and pretreatment is of great importance for obtaining correct results. Incorrect sampling may lead to unreliable results and, therefore, to the necessity for repeated sampling.

7.2.3 If biomaterial from several biotopes is required, repeat the procedure using new swab each time you collect biomaterial.

7.2.4 Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.

7.2.5 To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.

7.3. Sample collection

WARNING! Sample preparation may be required before DNA extraction!

7.3.1. Urine

Collect urine into a dry sterile 60 mL container with a hermetically screwed cap.

For the test, collect first portion of morning urine in amount of at least 20-30 mL.

Collecting residual urine after prostate massage

WARNING! If acute prostatitis is suspected, prostate massage is strictly forbidden.

Before collecting residual urine after prostate massage, at least three days of sexual abstinence are recommended.

The examinee urinates in the toilet leaving some urine in the bladder.

Before collecting urine, glans penis is treated with a sterile cotton swab moistened with saline solution.

The examinee undergoes prostate massage for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: with a soft prostate gland, light pressure is applied; with a dense consistency of the prostate, the pressure force is increased.

Residual urine is collected by the examinee after the massage is completed. The first portion of urine is collected in a dry sterile container with a volume of up to 60 ml, equipped with a hermetically sealed cap. in an amount of 10–15 ml.

After material collection, the container is tightly screwed and marked.

7.3.2. Prostate fluid

Before collecting prostate fluid, three days of sexual abstinence are recommended.

Before collecting the material, glans penis is treated with a sterile cotton swab moistened with saline solution.

Prostate secretions are collected after preliminary massage of the prostate gland through the rectum. The massage is performed by the doctor through an energetic pressing movement from the base to the top of the gland.

WARNING! If acute prostatitis is suspected, prostate massage is strictly forbidden.

7.3.3. Ejaculate

Before collecting ejaculate (semen), three days of sexual abstinence are recommended.

Before collecting the ejaculate, the examinee urinates in the toilet, completely emptying the bladder.

After urination, the examinee should wash his hands thoroughly with soap and toilet the external genitalia with soap and water. The glans penis and foreskin must be dried with a sterile tissue.

Ejaculate is obtained by masturbation. The ejaculate is taken into a sterile container with a volume of up to 60 mL, equipped with a hermetically screwed cap.

After material collection, the container is tightly screwed and marked.

7.3.4. Epithelial scrapes from urogenital tract

The material is collected using special medical products that have registration certificates, according to a procedure established depending on the source of the biological material.

WARNING! Use dry probe for biomaterial collection into the tubes with **PREP-RAPID** reagent. Avoid contacting skin, eyes and mucous membranes.

Method limitations[4]: local application of medicines, vaginal ultrasound, colposcopy less than 24 hours before the test.

Sampling is performed in accordance with the NA reagent kit instruction for use.

7.3.2.1 Urogenital scrapes sampling

Women on the day before the examination should not carry out genital toilet and sprays. In order to obtain an accurate result, it is necessary that the material to be examined contains as many epithelial cells as possible and a minimum amount of mucus and blood admixture.

WARNING! Before obtaining an epithelial scrape from the urethra, posterior vaginal vault and cervical canal, the free-flowing secretion should be removed with a sterile cotton swab.

If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material with a new probe into a new tube.

7.3.2.2 Vaginal sampling

The sample must be taken prior to physical examination. Speculum can be treated with warm water before the procedure. Antiseptics must not be used for speculum treatment. The sample must be taken from the lateral or posterolateral vaginal wall.

For virginal women, the sample must be taken from the vestibular mucous membrane, or, in special cases, from the posterior vaginal wall through the hymenal rings.

7.3.2.3 Urethral sampling

It is recommended to abstain from urinating for 1.5-2 hours before biomaterial sampling.

Immediately prior to sampling treat the external opening of the urethra with a tampon, which can be moistened with sterile saline solution.

In the presence of purulent discharge, it is recommended to take a scrape 15-20 minutes after urination, in the absence of discharge it is necessary to massage the urethra with a probe to take biomaterial. In women, the probe is inserted into the urethra to a depth of 1.0-1.5 cm, in children, the material for the test is taken only from the external opening of the urethra.

7.3.2.4 Cervical sampling

Remove mucus with a cotton swab prior to sampling and treat the cervix with sterile physiological saline solution. Carefully insert a sampling swab into the cervix to a depth of 0.5-1.5 cm. Avoid contact with the vaginal wall when removing the swab.

[4] - if it does not contradict the requirements to the NA extraction reagent kits being used

7.3.5. Rectal scrapes

The material is collected using special medical products that have registration certificates, according to a procedure established depending on the source of the biological material.

WARNING! Use dry probe for biomaterial collection into the tubes with **PREP-RAPID** reagent. Avoid contacting skin, eyes and mucous membranes.

Method limitations: rectal suppositories, laxatives, medications containing a high percentage of iron less than 48 hours before the test.

Sampling is performed in accordance with the NA reagent kit instruction for use.

7.3.6. Epithelial scrapes from oropharynx

The material is collected using special medical products that have registration certificates, according to a procedure established depending on the source of the biological material.

Scrapes are taken with a rotational movement from the surface of the tonsils, palatine arches and the posterior wall of the pharynx.

WARNING! Use dry probe for biomaterial collection into the tubes with PREP-RAPID reagent. Avoid contacting skin, eyes and mucous membranes.

Method limitations: topical use of medicinal products (sprays, drops, creams and ointments) less than 24 hours before the test; use of aerosols and other forms of medicinal products for inhalation in the treatment of bronchial asthma less than three hours before the test.

Sampling is performed in accordance with the NA reagent kit instruction for use.

7.3.7. Transport and storage of samples

Urine

Urine samples transport and storage conditions are determined by the NA extraction reagent kit instruction or instruction to the transport medium used for sample transport and storage.

Urine samples must be transported and stored (if it does not contradict the requirements for the NA extraction reagent kit):

- at temperature from 2 °C to 8 °C for no longer than 1 day;
- at temperature from minus 22 °C to minus 18 °C for no longer than one week.

WARNING! Only one freezing-thawing of material is allowed.

Scrapes from urogenital tract

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples.

It is allowed to store biomaterial according to the conditions indicated in Table 5 (if it does not contradict the requirements stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples):

Table 5. Biomaterial transport and storage conditions prior to DNA extraction

Biomaterial	Transport and storage temperature	Time period before DNA extraction
Urine	From 2 °C to 8 °C	Up to 24 hours
	From minus 22 °C to minus 18 °C	Up to 7 days
Prostate fluid Ejaculate Epithelial scrapes from urogenital tract Rectal scrapes Epithelial scrapes from oropharynx	From 2 °C to 8 °C	Up to 24 hours
	From minus 22 °C to minus 18 °C	Up to 1 month

WARNING! Only one freezing-thawing of material is allowed.

7.4. Sample preparation

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

8. PROCEDURE

DNA extraction from biological material

We recommend the authorized DNA extraction kits for the corresponding biomaterial types: **PREP-RAPID** (not recommended for male scrapes from urogenital tract), **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS**, **PREP-OPTIMA**, **PREP-OPTIMA MAX**, **PREP-MB-RAPID II**, **PREP-MB MAX** (Table 6).

DNA is extracted in accordance with the instruction to the NA extraction reagent kit.

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

Table 6. Kits recommended for DNA extraction (manufactured by “DNA-Technology”):

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-NA	Urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	50
PREP-NA PLUS	Urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	300
PREP-GS	Urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	100
PREP-GS PLUS	Urine, prostate fluid, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	300
PREP-RAPID	Urine, prostate fluid, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	500
PREP-OPTIMA, PREP-OPTIMA MAX	Urine, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes, epithelial scrapes from oropharynx	400

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-MB-RAPID II	Urine, epithelial scrapes from urogenital tract, epithelial scrapes from oropharynx	100
PREP-MB MAX	Urine, ejaculate, epithelial scrapes from urogenital tract, rectal scrapes	50

8.1. PCR preparation for package S

WARNING!

1. The reagents and tubes should be kept away from direct sun light.
2. When using package S, strips, strictly observe the completeness of the strips and caps. Do not use the caps for the strips of the other kits!
3. For package S, strips automated dosing is available using DTstream dosing instrument. Please refer to DTstream operation manual.

8.1.1 Mark one tube/stripped tube with the paraffin-sealed PCR mix for each test sample, negative control (C-), positive control (C+).

WARNING! The volume of reagents is calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 4 samples, mark 4 tubes for samples, one negative control tube “C-” and one positive control tube “C+”. Total number of tubes is 6.

8.1.2 Shake the tubes with Taq polymerase solution on vortex mixer for 3-5 seconds and spin in a vortex mixer for 1-3 seconds.

8.1.3 Add 10 µL of Taq polymerase solution to each tube. Avoid paraffin layer break.

WARNING! When using the Rotor-Gene Q detecting thermal cycler for PCR, no mineral oil is added to the tubes!

8.1.4 Add one drop (~20 µL) of mineral oil into each strip tube. Cover the tubes/strips loosely with caps.

8.1.5 Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS, PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If after extraction the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control “C-” on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

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

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-” tube. Avoid paraffin layer break.

8.1.8 Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.9 Spin the tubes/strips for 3-5 seconds in a vortex mixer (if using Rotor-Gene Q thermal cycler, spinning is not required).

8.1.10 Set the strips into the real-time thermal cycler.

8.1.11 **For DT thermal cyclers**: Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and IDs of the samples, positive and negative controls. Specify the position of the strips in the thermal unit and run PCR. See Table 7.

8.1.12 **For CFX96, Applied Biosystems QuantStudio 5 and Rotor-Gene Q detecting thermal cyclers**: Perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 8-10.

Table 7. The PCR program for DTlite, DTprime and DTprime II 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		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[7]		…	Holding		Holding
√ - optical measurement						

Table 8. The PCR program for CFX96 thermal cyclers (packages S, U)

Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 **√**	0:20	
√- optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex and Cy5 channels at 64 °C			

Table 9. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

Stage	Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	0:20	50
	2	62 **√**	0:20	
√- data collection for fluorophores (Fam, Vic (Hex), Cy5) is on				

[5] - Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[6] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

[7] - holding at 10 °C is allowed

Table 10. The PCR program for Rotor-Gene Q thermal cyclers (package S, tubes)

Cycling	Temperature	Hold Time	Cycle Repeats
Cycling 1	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	62 deg √	15 sec	
Cycling 3	94 deg	10 sec	45 times
	62 deg √	15 sec	
√– optical measurements, set Acquiring on Green, Yellow and Red detection channels at 62 °C.			

8.2. PCR preparation for package U, manual dosing

WARNING!

1. For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[8], sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.
2. The reagents and tubes should be kept away from direct sunlight.

8.2.1 Mark the required number of 0.2 mL tubes or a 96-well PCR microplate for each test sample, negative control (C-) and positive control (C+).

Note: It is recommended to test at least 5 samples per test (3 test samples, negative control and positive control).

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

8.2.2 Vortex the tube with PCR mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3 Add 6.0 µL of PCR mix to each tube/well (including “C-” and “C+”).

8.2.4 Vortex the tube with PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.2.5 Prepare the mixture of PCR buffer and TechnoTaq MAX polymerase. Add into the one tube:

6.0 x (N+1) µL of PCR buffer,
0.3 x (N+1) µL of TechnoTaq MAX polymerase,
where N is the number of marked tubes/microplate wells considering “C-”, “C+”.

Example: to test 4 samples, “C-” and “C+” (6 in total) in one PCR run, prepare the mixture of PCR buffer and Taq polymerase for 7 (6+1) tubes/wells, i.e. 42 µL of PCR buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin on vortex for 1-3 seconds to collect the drops.

WARNING! Mixture of PCR buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR buffer and TechnoTaq MAX polymerase mixture into each tube/well with PCR mix. Cover the tubes loosely.

[8] - 96-well PCR microplates are not used with DTlite detecting thermal cycler

WARNING! Follow the steps 8.2.8 – 8.2.14 within two hours after adding PCR buffer and TechnoTaq MAX polymerase mixture to PCR mix.

8.2.8 Vortex the tube with positive control "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS, PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If after extraction the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes/wells. Do not add DNA into the "C-", "C+" tubes/wells.

8.2.10 Add 6.0 µL of negative control ("C-") which passed whole DNA extraction procedure into the corresponding tube/well.

8.2.11 Add 6.0 µL of positive control sample ("C+") into the corresponding tube/well.

8.2.12 In case of using 96-well PCR microplates:

8.2.12.1. Place the PCR microplate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR microplate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.2.13 In case of using tubes:
Spin the tubes for 3-5 seconds on vortex mixer.

8.2.14 Set the tubes/PCR microplate into the real-time thermal cycler.

8.2.15 **For DT thermal cyclers**: Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 11.

8.2.16 **For CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers**: Perform PCR considering the volume of reaction mixture of 18 µL. See Tables 8, 9.

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

Table 11. The PCR program for DTlite, DTprime and DTprime II thermal cyclers for package U

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	25[11]			Holding		Holding
√ - optical measurements						

8.3. PCR preparation for package U, using DTstream (only for DTprime *X*, DTprime II *X* thermal cyclers)

WARNING!

1. For amplification use 384-well microplates hermetically sealed with thermal film.
2. The reagents and tubes should be kept away from direct sun light.

Note. It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.3.1 Vortex the tube with PCR mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.3.2 Vortex the tube with PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! TechnoTaq MAX polymerase should be taken out from the freezer immediately prior to use.

8.3.3 Prepare the mixture of PCR buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.3.4 Vortex the tube with the mixture PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.5 Vortex the tubes with positive control for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS, PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

[11] - holding at 10 °C is allowed

3. In case of using **PREP-MB MAX** DNA extraction kit, carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If after extraction the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control “C-” on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

8.3.6 Set the tubes with PCR mix, the mixture of PCR buffer and TechnoTaq MAX polymerase, tubes or deep-well plates with DNA samples, positive and negative controls and PCR microplate on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the working table of DTpack sealing device.

8.3.8 Run the process of sealing of PCR microplate according to the user manual of DTpack sealing device.

8.3.9 Centrifuge the microplate at RCF(g) 100 for 30 seconds.

8.3.10 Set the PCR microplate into the real-time thermal cycler.

8.3.11 Launch the operating software for DT instrument[12]. Add corresponding test[13], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit and run PCR. See Table 11.

9. CONTROLS

The **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit** contains positive control. 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.

WARNING! A negative 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 indicated in supplied instructions.

For **HSV1/HSV2 Multiplex 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 considered;
- the exponential growth of the fluorescence level is absent for the specific product and is present for internal control.

For **HSV1/HSV2 Multiplex 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 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.

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

10. DATA ANALYSIS

10.1 Registration of the results is carried out automatically during amplification by the software provided with detecting thermocycler.

10.2 For CFX96 (Bio-Rad) detecting thermal cyclers, it is recommended to use the regression type of analysis (Cq Determination Mode: Regression) and select “Baseline Subtraction Curve Fit” in the tab “Baseline Setting”.

10.3 The results are interpreted according to Table 12. The run results are valid if the conditions for interpreting the results obtained for the controls are fulfilled.

Table 12. PCR results interpretation

Detection channel			**Result interpretation**
Fam/Green, Cp/Cq/Ct	Hex/Yellow/Vic, Cp/Cq/Ct	Cy5/Red, Cp/Cq/Ct	
Test samples			
Specified	Not considered	Not specified	***Herpes simplex virus 2 (HSV2)*** **DNA is detected**
Not specified	Not considered	**Specified**	***Herpes simplex virus 1 (HSV1)*** **DNA is detected**
Not specified	**Specified**	Not specified	DNA of the target microorganisms is not detected
Not specified	Not specified	Not specified	Invalid result
Negative control			
Not specified	**Specified**	Not specified	**Negative result** Run results are valid
Positive control			
Specified	**Specified**	**Specified**	**Positive result** Run results are valid

10.4 An invalid result may be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect performance of the test protocol; noncompliance with the amplification temperature regime, etc. In this case, PCR with the available DNA preparation needs to be repeated, or the DNA must be extracted and PCR performed again, or the biological material must be taken again (performed sequentially).

10.5 Cp/Cq/Ct values obtained for the biological sample on Fam or Cy5 detection channels less than 24 indicate high concentration of the corresponding microorganism’s DNA. In this case a false negative result is possible for the microorganism whose DNA is present in low concentration. To eliminate the possibility of false-negative results, we recommend to repeat PCR of the extracted DNA preparation using “DNA-Technology” made **Herpes simplex virus 1, 2 REAL-TIME PCR Detection Kit.**

10.6 If positive result is obtained for negative control, the results of the whole run are considered unreliable. In this case, special measures for identification and elimination of possible contamination are required.

10.7 If negative result is obtained for positive control, the results of the whole run are considered unreliable. In this case, a repeated amplification of the whole batch of samples is required.

11. SPECIFICATIONS

a. Analytical specificity

For human biomaterial samples containing the DNA of the detected microorganisms, the detecting thermal cycler software registers positive amplification results for the specific products (*Herpes simplex virus 1, Herpes simplex virus 2* genome fragments) on the corresponding detection channel.

For human biomaterial samples not containing the DNA of *Herpes simplex virus 1* or *Herpes simplex virus 2*, the detecting thermal cycler software registers negative amplification results for the specific products on the corresponding detection channels and positive result for the internal control (IC) on Hex/Yellow/Vic detection channel.

The absence of nonspecific positive amplification results has been shown in high DNA concentrations of: *Cytomegalovirus*, *Varicella-zoster virus, Epstein-Barr virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Staphylococcus spp., Streptococcus spp., Parvovirus B19, Chlamydia pneumonia, Mycoplasma hominis, Trichomonas vaginalis, Neisseria gonorrhoeae, Toxoplasma gondii, Mycoplasma genitalium, Chlamydia trachomatis, Gardnerella vaginalis* and human DNA in concentration up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity

Limit of detection amounts to 5 copies of each microorganism DNA per amplification tube.

Sensitivity is determined by the analysis of serial dilutions of two batches of the laboratory control (LC).

Limit of detection in test sample depends on the used DNA extraction kit and the amount of the extracted DNA preparation obtained:

Biomaterial	**DNA extraction reagent kit**	**Obtained preparation, µL**	**Limit of detection, copies per sample**
Urine (extraction from 1.0 mL of sample)	PREP-NA PREP-MB MAX	50	50
	PREP-GS PREP-MB-RAPID II	100	100
	PREP-NA PLUS PREP-GS PLUS	300	300
	PREP-OPTIMA PREP-OPTIMA MAX	400	400
	PREP-RAPID	500	500
Prostate fluid (extraction from 100 µL of sample)	PREP-NA	50	50
	PREP-GS	100	100
	PREP-NA PLUS PREP-GS PLUS	300	300
	PREP-RAPID	500	500
Epithelial scrapes in transport medium	PREP-NA PREP-MB MAX	50	50
	PREP-GS PREP-MB-RAPID II	100	100
	PREP-NA PLUS PREP-GS PLUS	300	300
	PREP-OPTIMA PREP-OPTIMA MAX	400	400
	PREP-RAPID	500	500
Ejaculate (extraction from 100 µL of sample)	PREP-NA PREP-MB MAX	50	50
	PREP-GS	100	100

Biomaterial	DNA extraction reagent kit	Obtained preparation, µL	Limit of detection, copies per sample
	PREP-NA PLUS PREP-GS PLUS	300	300
	PREP-OPTIMA PREP-OPTIMA MAX	400	400

c. Diagnostic characteristics

Biomaterial type	Analyte	Diagnostic sensitivity	Diagnostic specificity
Epithelial scrapes from urogenital tract	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Urine	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Prostate fluid	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Ejaculate	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Rectal scrapes	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Epithelial scrapes from oropharynx	HSV1	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	HSV2	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Total	100% (95% CI: 98.78% – 100%)	100% (95% CI: 98.78% – 100%)

d. Precision and repeatability

Precision amounts to 100%.

Repeatability amounts to 100%.

Note. The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS**, **PREP-OPTIMA, PREP-OPTIMA MAX, PREP-RAPID, PREP-MB-RAPID II** and **PREP-MB MAX** extraction kits.

12. TROUBLESHOOTING

Table 13. 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 encounter any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

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

13. QUALITY CONTROL

The quality controlprocedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our customer service with quality issues of **HSV1/HSV2 Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for<n>tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Non-sterile