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IVD

For professional use only

CMV REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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REF

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R1-P204-23/9EU

R1-P204-UA/9EU

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1. INTENDED USE

The **CMV REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **CMV REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) for qualitative pathogen detection. The **CMV REAL-TIME PCR Detection Kit** is designed to detect CMV nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: saliva, urine, prostate fluid, ejaculate, swabs from urethra and conjunctiva of the eye, cervix, or posterolateral vaginal wall, breast milk, peripheral blood mononuclear cells, liquor, amniotic fluid, tissue samples.

CMV infection is typically unnoticed in healthy people, but can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or newborn infants. After infection, CMV remains latent within the body throughout life and can be reactivated at any time. Eventually, it may cause mucoepidermoid carcinoma and possibly other malignancies such as prostate cancer.

Congenital CMV is the leading infectious cause of deafness, learning disabilities, and mental retardation in children. CMV also seems to have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality.

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

The **CMV 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 or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin is melted or thermal dissociation of a complex of Taq polymerase and antibodies is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **CMV 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 CMV product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of two fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. 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
CMV	IC	-	-	-

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers (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 **CMV REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) real-time thermal cyclers.

3. CONTENT

The detailed description of content is represented in the Tables 2 - 3.

Table 2. The **CMV REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P204-23/9EU and R1-P204-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes or 12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps[1]	12 8-caps		

Table 3. The **CMV REAL-TIME PCR Detection Kit** content, package U (universal) for R1-P204-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or slightly 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 kit is intended for single use and designed for 96 tests (no more than 94 defined samples, one positive control and one negative control) for package S.

Package U is designed to carry out 96 tests if at least 5 samples in one study are amplified (3 unknown samples, positive and negative control samples).

[1] - for detection kit packaged in strips REF R1-P204-S3/9EU

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, single use 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 physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Physiological saline solution 0.9% NaCl (Sterile);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID** REF P-001/1EU (not applicable to male urethral swabs), **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU extraction kits are recommended);
- High speed centrifuge (RCF(g) not less than 16000);
- Solid-state thermostat (temperature range 50-98°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- 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;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 50 µL, 200 µL, 1000 µL);
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips.

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. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **CMV REAL-TIME PCR Detection Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C during 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 TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

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 TechnoTaq MAX polymerase 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;
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

An expired **CMV 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 **CMV REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **CMV 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. 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, reverse transcription, 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.

Do not open the tubes after amplification. 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. 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 instruction to use.

7. SAMPLES

The **CMV REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from saliva, urine, prostate fluid, ejaculate, swabs from urethra and conjunctiva of the eye, cervix, or posterolateral vaginal wall, breast milk, peripheral blood mononuclear cells, liquor, amniotic fluid, tissue samples, depending on professional prescription.

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 amplification.

PCR inhibitors are the presence of mucus, blood impurities, lubricants, talc, local medicines.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control sample 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.

Following medicines have no effect on the amplification of the laboratory control sample and internal control: chlorhexidine bigluconate – 5%, Miramistin ® - 5%.

Impurities contained in the biomaterial sample are almost completely removed during the DNA extraction. 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. It is not recommended to use express methods of DNA extraction.

The features of genitourinary swabs sampling:

Women should not carry out genitals toilet and vaginal douching the day before research. To obtain an objective result, it is necessary that the material contains the largest count of epithelial cells and the minimum amount of mucus and blood impurities. Incorrect intake of biological material can lead to uncertain results and, therefore, to re-sample of biomaterial.

The features of the posterior vaginal vault sampling:

The material should be taken before the physical inspection. The speculum before manipulation can be moistened with hot water, the use of antiseptics for speculum treatment is contraindicated. Scraping is taken from the posterior vaginal vault. In case of virginal women, scraping is taking from the vestibular mucous membrane, and in some cases from the posterior vaginal vault through hymenal rings.

The features of the urethral sampling:

Before sampling procedure, the patient is recommended to refrain from urination for 1.5 – 2 hours.

Immediately before sampling procedure, it is necessary to treat the external urethral orifice with a tampon moistened with sterile physiological solution.

In the presence of purulent discharge, the sample must be taken 15-20 minutes after urination. In the absence of discharge, it is necessary to massage the urethra with sampling swab or brush. In case of

women, the swab or brush is inserted to a depth of 1.0-1.5 cm, in case of children, the material is taken only from the external urethral orifice.

The features of the cervical sampling:

Before sampling procedure, it is necessary to remove the mucus with a cotton tampon and, then, treat the cervix with a sterile physiological solution. The sampling swab is inserted into the cervical canal to a depth of 0.5 – 1.5 cm. Removing the swab, contact of the walls of the vagina should be excluded.

The features of the eye conjunctiva sampling:

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 probe.

Genitourinary swabs sampling (cervical canal, vagina, urethra) and eye conjunctiva sampling

Procedural limitations - local application of medicines, vaginal ultrasound less than 24 hours before the procedure.

Sampling procedure is carried out using special sterile disposable instruments – urogenital swabs, cytobrushes or tampons, depending on the source of clinical material in accordance with established procedures.

ATTENTION! In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the scrapes is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a sterile physiological solution;
- in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR;
- in tubes with **PREP-RAPID** (manufactured by “DNA-Technology Research&Production”, LLC).

ATTENTION! PREP-RAPID is not recommended for DNA extraction from male urogenital scrapes.

Order of taking:

1. Open the tube.

2. Move the swab with biological material to the tube with physiological solution, transport medium, or **PREP-RAPID**, and rinse it thoroughly, avoiding splashing of the liquid. Then, remove the swab from the solution, pressing it to the wall of the tube, press out the excess liquid, remove the swab and discard. In the case of taking biomaterial from several biotopes, repeat the procedure, taking the material with a new swab into a new tube each time.

3. Tightly close the tube, mark the tube.

ATTENTION! Samples may be stored at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. In case of usage transport media biological material samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Pretreatment, sampling and storage of the material is carried out in accordance with the instruction for use for DNA extraction kit.

4. In case of taking the swabs in tubes with physiological solution or transport medium, it is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** and **PREP-MB RAPID** kits:

4.1 The tube containing the sample shall be centrifuged at RCF(g) 16000 for 10 minutes at room temperature between 18 °C and 25°C.

NOTE - Use a centrifuge for 1.5 mL tubes with RCF(g) no less than 16000, for example, HERAEUS pico17 centrifuge (RCF(g) 17000).

4.2 Remove the supernatant. Using **PREP-GS**, leave approximately 50 µL in tube (precipitate + liquid fraction). Using **PREP-NA** and **PREP-MB RAPID**, leave 100 µL (precipitate + liquid fraction). Tightly close the tubes.

The resulting material is ready for DNA extraction.

Taking swabs in tubes with the **PREP-RAPID**, pretreatment is not required. The material is ready for DNA extraction.

The first portion of morning urine

The first portion of the morning urine as a biological material is used in acute inflammation of the lower urinary tract due to pain of taking scraping epithelial cells.

The first portion of morning urine in the amount of 10–15mL is selected for the analysis. It is possible to examine the first portion of urine received 2 or more hours after the previous urination.

The urine is taken into a special dry sterile container with a volume of up to 60 mL, equipped with a hermetically screw-cap.

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

The prostate fluid

Before taking the prostate fluid, sexual abstinence is recommended for 3 days before the procedure.

Before taking the prostate fluid, the penis balanus is treated with a sterile cotton tampon moistened with a physiological solution.

The prostate fluid is collected after a prostate massage through the rectum. Massage is performed by a doctor, by means of vigorous pressing movement from the base to the top of the gland.

After the end of the massage, the released prostate fluid in the form of a free flowing drop (0.15-1.0 mL) is collected in a 2 mL single dry sterile tube or a container with a volume of up to 60 mL.

The container with the prostate fluid is hermetically screwed and marked.

ATTENTION! Suspecting acute prostatitis, the prostate massage is strictly prohibited!!!

Residual urine after prostate massage

Before residual urine after prostate massage, sexual abstinence is recommended for 3 days before the examination.

The patient urinates in the toilet, leaving part of the urine in the bladder.

Before urine taking, the penis balanus is treated with a sterile cotton tampon moistened with a physiological solution.

The prostate massage is carried out for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: with a soft prostate - slight pressure is carried out, with a dense consistency of the prostate - the pressure force is increased.

After the end of the massage, the first 10-15 mL of the urine is collected in a sterile container with a volume of up to 60 mL.

Container is tightly screwed and marked.

ATTENTION! Suspecting acute prostatitis, the prostate massage is strictly prohibited!!!

Ejaculate

Before collecting ejaculate (seminal fluid), sexual abstinence is recommended for 3 days before the examination.

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

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

The ejaculate is obtained by masturbation and collected in a sterile container with a volume of up to 60 mL.

The container with ejaculate is hermetically closed and marked.

Saliva, liquor, amniotic fluid

1. Saliva, liquor, amniotic fluid (about 500 µL) is collected in a sterile container and tightly closed.
2. Then 500 µL of the material is transferred into a 1.5 mL tube.
3. The tube is centrifuged at RCF(g) 13000 for 10 minutes at room temperatures from 18 °C to 25 °C.
4. The supernatant is removed, leaving 50 µL in tube (precipitate + liquid fraction).
5. 500 µL of a sterile saline solution is added to the precipitate.
6. The tube is centrifuged at RCF(g) 13000 for 10 minutes at room temperatures from 18 °C to 25 °C.
7. The supernatant is removed, leaving 100 µL in tube (precipitate + liquid fraction).

Breast milk

Material is collected into a sterile container and tightly closed.

The material is gently shaked and 1.0 mL of the material is transferred into a 1.5 mL tube.

Note. The milk collection period is not more than 24 hours. Storage for the entire collection period at temperatures from 2 °C to 8 °C.

Peripheral/umbilical cord blood

Peripheral/cord blood sampling is carried out in vacuum tubes. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example disodium 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 turning the tube 2 – 3 times.

Tissue samples

Pieces of the tissue (diameter is not more than 5 mm) are placed in sterile 2 mL tubes with appropriate transport medium. The test tube is tightly closed and marked.

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

NOTE - 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 kit's instruction for use.

8. PROCEDURE

DNA extracting from biological material.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID** and **PREP-MB RAPID** extraction kits are recommended. **PREP-RAPID** is not recommended for DNA extraction from men urogenital swabs.

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

8.1 Assay procedure for package S:

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

ATTENTION! When using package S, strips, strictly observe the completeness of the strips and caps for them. Do not use caps for strips from other kits!

8.1.1 Mark tubes with PCR-mix for each test sample, negative control (C-) and positive 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.

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

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

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

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

ATTENTION! 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.

ATTENTION! In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. 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.

ATTENTION! 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.

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

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-“ tube and positive control (C+) into corresponding tube. Avoid paraffin layer break. Spin the tubes for 3-5 seconds.

8.1.8 Spin tubes/strips for 3-5 seconds.

8.1.9 Set the tubes/strips into the Reai-time Thermal Cycler.

8.1.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], 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 (see 8.1.9) and run PCR. See Tables 4, 8.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 5-8.

8.2 Assay procedure for package U:

8.2.1 Mark the required number of 0.2 mL tubes for each sample to be tested, for positive control (C+) and for negative control (C-).

Example: to test 4 samples in one PCR run, mark 4 tubes for samples, 1 tube for "C-" and 1 tube for "C+". The resulting number of tubes is 6.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds and spin down drops for 1-3 seconds.

8.2.3 Add 6.0 µL of PCR-mix into the each marked tube for samples to be tested.

8.2.4 Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin for down drops for 1-3 seconds.

ATTENTION! TechnoTaq MAX polymerase must be stored at temperatures from minus 18°C to minus 22°C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase.

Add into one tube:

- 6,0×(N+1) µL of PCR-buffer,
- 0,3×(N+1) µL of TechnoTaq MAX polymerase,

N — number of the marked tubes including "C-" and "C+".

Example: for simultaneous testing of 4 samples, "C-" and "C+" in one PCR run, mark 6 tubes (4 tubes for samples to be tested, 1 tube for "C+" and 1 tube for "C-"). Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase for 7 (6+1) tubes. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with prepared mixture for 3-5 seconds, then spin down drops for 1-3 seconds.

ATTENTION! The mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared just prior to use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each PCR-tube. Close tubes.

ATTENTION! Follow the steps listed in pp 8.2.8. – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to amplification mix.

8.2.8 Vortex the tubes with sample, "C-" and "C+" for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! 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.

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

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

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. 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.

ATTENTION! Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding PCR-tubes. Do not add DNA into the "C-" and "C+" tubes. Avoid paraffin layer break.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube and positive control (C+) into corresponding tube. Avoid paraffin layer break. Close the tubes tightly.

8.2.11 Spin tubes for 3-5 seconds.

8.2.12 Set the tubes into the Real-time Thermal Cycler.

8.2.13 Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.2.12) and run PCR. See Tables 8-12.

8.3 Assay procedure for package U using DTstream:

8.3.1. Vortex the tube with PCR-mix for 3-5 seconds and spin down drops for 1-3 seconds.

8.3.2. Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! TechnoTaq MAX polymerase must be stored at temperatures from minus 18°C to minus 22°C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

8.3.3. Following the DTstream software instructions, prepare a mixture of PCR-buffer with TechnoTaq MAX polymerase in a separate test tube.

8.3.4. Vortex the tube with prepared mixture for 3-5 seconds, then spin down drops for 1-3 seconds.

8.3.5. Vortex the tubes with samples, "C-" and "C+" for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! 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**, vortex the tubes for 3-5 seconds on a vortex mixer, put the tubes with the DNA preparation in magnetic rack and transfer the supernatant containing the isolated DNA to new tubes. If, after DNA extraction, the supernatant containing the isolated DNA was already transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

8.3.6. Set tubes with PCR-mix, PCR-buffer and TechnoTaq MAX polymerase mixture, DNA sample, positive control and negative control and microplate for PCR to the DTstream and dispense the components according to the instruction manual.

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

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

8.3.7. After completion of the program on the DTstream, set gently, without shaking, the microplate to the DTpack.

8.3.8. Carry out the procedure of sealing the microplate by thermal film in accordance with the instructions to the DTpack.

8.3.9. Spin the microplate at RCF(g) 1000 for 30 seconds.

8.3.10. Set the microplate into the Real-time Thermal Cycler.

8.3.11. Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.3.10) and run PCR. See Tables 8-12.

Table 4. 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	…	…	Holding		Holding

Table 5. 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

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

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

Table 6. 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 7. The PCR program for Rotor-Gene 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 8. Detection channels

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

Table 9. 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	5	15		Cycle
	94	0	5			
2	94	5	0	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		v	
4	94	0	10	45		Cycle
	64	0	15		v	
5	94	0	5	1		Cycle
6	10	…	…	Holding		Holding

Table 10. 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	5 min	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

Table 11. 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	5 min	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 12. The PCR program for Rotor-Gene thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 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	

9. CONTROLS

The **CMV REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the CMV 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.

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

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 absent and for internal control is present.

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 performed automatically. In other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing CMV 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 CMV 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 instruction for use). The cutoff Ct values for Rotor-Gene 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 **CMV 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 CMV DNA are to be registered positive for specific product (a fragment of the CMV genome). The samples free of CMV DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification DNA sample in the presence of Herpes simplex virus 1, 2, Human herpesvirus 6, 8, HPV 6, 11, Epstein Barr virus, Varicella zoster virus, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, *Neisseria gonorrhoeae*, *Candida albicans*, *Streptococcus sp*., *Staphylococcus sp*., 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 **CMV 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 CMV 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
10	94	94	100
5	94	93	98,9
2	94	73	77.7
1	94	56	59.6
0	94	0	0

Sensitivity of CMV 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 CMV 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
- scraping of epithelial cells in 500 µL transport medium; - ejaculate in 500 µL transport medium; - prostate fluid in 500 µL of transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample	300 copies /sample	500 copies /sample

c. Diagnostic characteristics

Number of samples (n) - 424;

Diagnostic sensitivity (95% CI) - 98.0% (91.8-98.0%);

Diagnostic specificity (95% CI) – 100% (99.2-100%).

NOTE - 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/8EU kits.

12. TROUBLESHOOTING

Table 13. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	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 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

“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.

Contact our customer service with quality issues of **CMV REAL-TIME PCR Detection Kit**:

Technical support:

E-mail: hotline@dna-technology.ru

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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
	Do not use reuse		Caution
EC REP	Authorized representative in the European Community		

REF

R1-P204-S3/9EU

R1-P204-23/9EU

R1-P204-UA/9EU

VER

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