DNA-TECHNOLOGY

1487 2026-05-25

IVD

For professional use only

HPV-QUANT-21® quantitative PCR Detection Kit

INSTRUCTION FOR USE

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REF R1-P317-S3/5EU

VER 1487.2026.05.25

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1. INTENDED USE

The **HPV-QUANT-21® quantitative PCR Detection Kit** is intended for research and diagnostic applications. The **HPV-QUANT-21® quantitative PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HPV-QUANT-21® quantitative PCR Detection Kit** is designed to detect HPV nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial cell swabs, prostate fluid, ejaculate, urine, biopsy material.

The **HPV-QUANT-21® quantitative PCR Detection Kit** is *in vitro* DNA test, which is intended for the specific identification and quantification low-risk human papilloma virus types (HPV 6, 11, 44) and high-risk human papilloma virus types (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) in human biological samples.

Indications for the use: preventive HPV screening for cervical precancer and cancer, monitoring of patients with HPV infection, diagnosis of HPV infection in oncopathology of the cervix, penis, prostate, anus, larynx, inflammatory diseases of the genitourinary system in women and men.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HPV QUANT-21® quantitative PCR Detection Kit.**

The **HPV-QUANT-21® quantitative 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 **HPV-QUANT-21® quantitative PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter fluorescent dyes 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.

One tube contains a PCR-mix for the amplification of human genomic DNA (sample intake control (SIC)). The SIC allows to exclude preanalytical error. If the amount of collected material is insufficient for the analysis, it is necessary to repeat sampling procedure.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probes used for the detection of the HPV product amplification include fluorescent dyes Fam, Rox and Cy5. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The Fam dye label is also used for SIC detection.

Defined tubes contain additional probe with Rox dye label – “Marker”. It tags the strip orientation. Upon completion of run, software defines actual position of the strip (by means of “marker” position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

№ of the tube in a strip	Dye label/detection channel				Color labeling of the PCR-mix
	Fam	Hex	Rox	Cy5	
1	HPV **31** type	IC	HPV **35** type	HPV **16** type	Blue
2	HPV **52** type	IC	HPV **33** type	HPV **68** type	Colorless
3	HPV **45** type	IC	HPV **82** type	HPV **51** type	
4	HPV **6** type	IC	HPV **44** type	HPV **11** type	
5	HPV **18** type	IC	HPV **39** type	HPV **58** type	
6	HPV **66** type	IC	HPV **26** type	HPV **53** type	
7	HPV **59** type	IC	HPV **56** type	HPV **73** type	
8	SIC	IC	Marker	-	

The quantification of HPV DNA is possible by 2 types of analysis: absolute and relative. Absolute analysis involves software calculation of the virus copies number based on the value of the threshold cycle (Cp) after amplification. Relative analysis involves normalizing the virus DNA amount to the amount of human genomic DNA (SIC) in a given sample (i.e., the number of human cells in the sample). It allows to consider for the sampling variance.

⚠ Clinically significant virus concentration is at least 10^3 copies of HPV DNA per 10^5 human cells (with correct material sampling). This value characterizes the high infection level and can lead to the development of cervical neoplasia. Therefore, software restriction of the obtained virus concentration values is used during data analysis if they do not fall within the clinically significant range.

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **HPV-QUANT-21® quantitative PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

3. CONTENT

The **HPV-QUANT-21® quantitative PCR Detection Kit** contains PCR-mix, MAX Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **HPV-QUANT-21® quantitative PCR Detection Kit** content, package S (standard) for R1-P317-S3/5EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or blue transparent liquid under waxy white fraction	3840 µL (20 µL in each tube)	24 8-tube strips
MAX Taq-polymerase solution	Colorless transparent liquid	2000 µL (500 µL in each tube)	4 tubes
Mineral oil	Colorless transparent viscous oily liquid	4.0 mL (1.0 mL in each tube)	4 tubes
Positive control	Colorless transparent liquid	160 µL	1 tube
Strip's caps	24 8-caps		

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

The kit is intended for single use and designed for 24 tests for **HPV-QUANT-21® quantitative PCR Detection Kit.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: sterile single use swabs, cytobrushes, cotton swabs e.t.c for sampling of biomaterial;
- Sterile containers to collect clinical material;
- Sterile tubes containing transport medium: “DNA-Technology” made **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;
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-NA PLUS** (REF P-002/2EU) or **PREP-GS PLUS** (REF P-003/2EU) and **PREP-MB RAPID** (REF P-116-A/8EU) extraction kits are recommended)**;**
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-65 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Single channel pipettes (dispensers covering 1.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for strips;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes in strips;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;

– Disinfectant solution.

Post-Amplification – Amplification detection area:

– Real-time PCR thermal cycler.

Software:

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

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

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **HPV-QUANT-21® quantitative PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C over the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

Transportation is allowed in thermal containers with icepacks by all types of covered transport at temperatures from 2 °C to 8 °C inside the container over the transportation or at temperatures from 2 °C to 25 °C inside the container, but for no longer than 5 days.

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

– components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;

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

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

An expired the **HPV-QUANT-21® quantitative 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 **HPV-QUANT-21® quantitative PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **HPV-QUANT-21® quantitative PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

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

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal

protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

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

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

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

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

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

Emergency actions

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

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

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

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

Do not use the kit:

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

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

7. SAMPLES

The **HPV-QUANT-21® quantitative PCR Detection Kit** is designed to detect DNA extracted from the epithelial swabs, prostate fluid, ejaculate, urine, biopsy samples depending on professional prescription.

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

Interfering substances

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

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

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.

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.

General requirements

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

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.

Professional prescription is required to localize the place of sampling. The decision must be based on a patient's complaints and clinical signs, and made by the physician in charge.

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.

Genitourinary swabs

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 vaginal sampling using a device for self-sampling

The sampling is carried out in accordance with the instructions for use of the device.

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 cervix uteri sampling

The sample must be taken prior to physical inspection.

Before taking the material, remove the mucus, inflammatory exudate or blood (if any) with a sterile cotton swab.

The exfoliative cellular material and the superficial epithelium should be carefully scraped off from the vaginal portion of the cervix, the area of the transformation zone (CT) and/or the cervical canal if the connection zone of the stratified squamous epithelium and cylindrical epithelium is moved into the cervical canal.

Urogenital swabs sampling (cervical canal, cervix uteri, vagina, urethra)

Procedural limitations - local application of medicines, vaginal ultrasound less than 24 hours before the procedure. Women must not perform hygiene procedures or syringing prior the sampling 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. The sampling using a device for self-sampling is carried out in accordance with the instructions for use of the device.

 In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the swabs 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.

Order of taking:

1. Open the tube.

2. Scrape epithelial cells from the corresponding biotope (i.e. vagina, urethra, cervical canal, anus, oropharynx) with a sterile swab.

3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.

4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.

5. Close the tube tightly and mark it.

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 user manual for DNA extraction kit.

Preparation of the vaginal material taken by using a device for self-sampling

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

1. Add 500 µL of saline solution into the tube (or other container specified in the instruction for use of the device for self-sampling (further - tube)) with the device tip.
2. Vortex the tube (15 seconds).
3. Remove the device tip from tube and throw out.
4. Transfer into 1.5 mL new tube.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA PLUS** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS PLUS**. Tightly close the tubes.

The resulting material is ready for DNA extraction.

The first portion of morning urine

The first portion of morning urine in the amount of 20–30 mL is selected for the analysis.

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.

Preparation of the urine material

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

1. Transfer 1.0 mL of the material from container into 1.5 mL plastic tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of the sterile saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving 100 µL in tube (precipitate + liquid fraction) using **PREP-NA PLUS** and **PREP-MB RAPID** or 50 µL in tube (precipitate + liquid fraction) using **PREP-GS PLUS**. Tightly close the tubes.

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 2.0 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.

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.

Biopsy samples

The biopsy samples (bioptat) are placed into a sterile tube with sterile physiological saline solution or water (no more than 5.0 mL) or into an empty sterile tube.

The test tube is tightly closed and marked.

Transportation and storage of the samples

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

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

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

8. PROCEDURE

DNA extracting from biological material

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

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

Assay procedure

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

Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1 Mark the required number of strips with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-).

One strip contains PCR-mixes for one sample testing.

Example: to test 2 samples, mark 2 strips for test samples, 1 strip for “C+” and 1 strip for “C-”. The resulting number of strips is 4.

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

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

8.4 Add one drop (~20 µL) of mineral oil into each tube of the strip. Close strips tightly.

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

In case of using **PREP-GS PLUS 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 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 3-5 seconds in a vortex mixer.

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

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

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

8.8 Spin strips for 3-5 seconds.

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

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

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

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

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10[1]	…	…	Holding		Holding

√ - optical measurement
[1] – holding at 25°C is allowed

9. CONTROLS

The **HPV-QUANT-21® quantitative PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the HPV 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. The PCR-Mix contains sample intake control (SIC). Sample intake control (SIC) estimates the amount of human DNA in the tube. When estimating the relative number of HPV, the SIC value is used for normalization. To reveal possible contamination a negative control is required.

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:

- Positive result for the specific product is present, in this case the internal control is not taken into account. In the presence of HPV DNA in the test sample, the absolute quantity of this virus type (the degree of concentration common logarithm, number of copies of the HPV DNA per sample) will be specified in the line with the name of this type of HPV in the “Quantitative” field (absolute analysis).
- Positive result for the specific product is absent and for internal control is present.

SIC value must be considered when analyzing results: SIC values lower than 4.0 should be considered as an insufficient amount of sample, and the sampling procedure must be repeated.

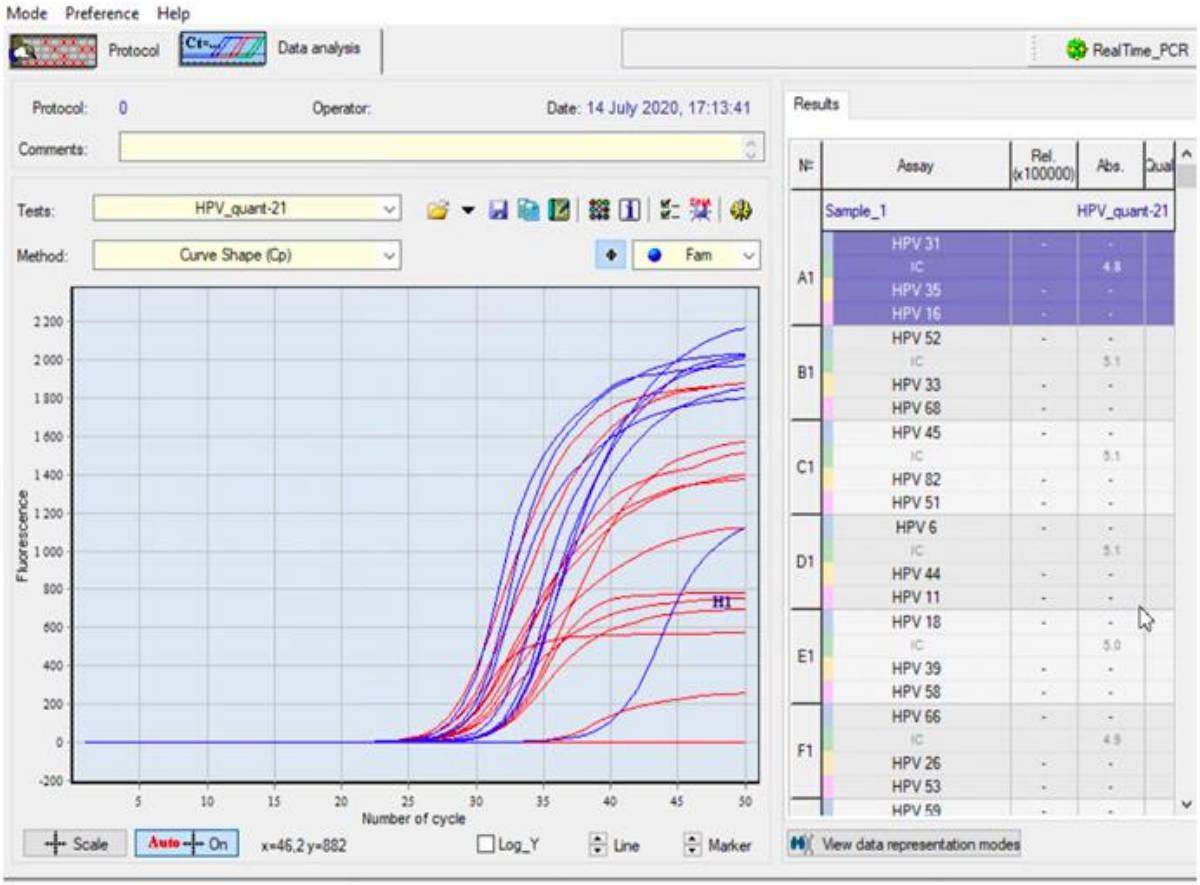
The test result is considered invalid when a positive result for the specific product and for internal control is absent.

If positive control (C+) has **not** positive result for the specific product, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

In case of obtaining positive result for negative control sample all results of the current PCR run are considered false. In this case conduction of special procedures against possible contamination is required.

10. DATA ANALYSIS

Registration and interpretation of the PCR results are held in automatic mode. Analysis will be performed by Real-Time PCR application. The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube. Type of the sample, name of the test, value of the threshold cycle (Cp) and test result (relative, absolute and quantitative) will be displayed in the right module of the window. Operator can create, save and print a report.



HPV

HPV_quant-21

Date 14 July 2020, 17:13:41
Number of tube ---
Patient name ---
Sex ---
Age ---
Organization ---
Clinician name ---
Comments ---

Information about laboratory

Sample ID: Sample_3

№	Name of research	Results		
		Relative, Lg(X/SIC)*	Quantitative, Lg(copies/sample)	Qualitative
1	HPV 31	5.5	7.5	
2	HPV 35	not detected	not detected	
3	HPV 16	not detected	not detected	
4	HPV 52	not detected	not detected	
5	HPV 33	not detected	not detected	
6	HPV 68	not detected	not detected	
7	HPV 45	not detected	not detected	
8	HPV 82	not detected	not detected	
9	HPV 51	not detected	not detected	
10	HPV 6	not detected	not detected	
11	HPV 44	2.1	4.1	
12	HPV 11	not detected	not detected	
13	HPV 18	not detected	not detected	
14	HPV 39	not detected	not detected	
15	HPV 58	not detected	not detected	
16	HPV 66	not detected	not detected	
17	HPV 26	not detected	not detected	
18	HPV 53	not detected	not detected	
19	HPV 59	not detected	not detected	
20	HPV 56	not detected	not detected	
21	HPV 73	not detected	not detected	
22	SIC		5.0	

* HPV DNA copies/10^3 cells (Lg)

Study was carried out by

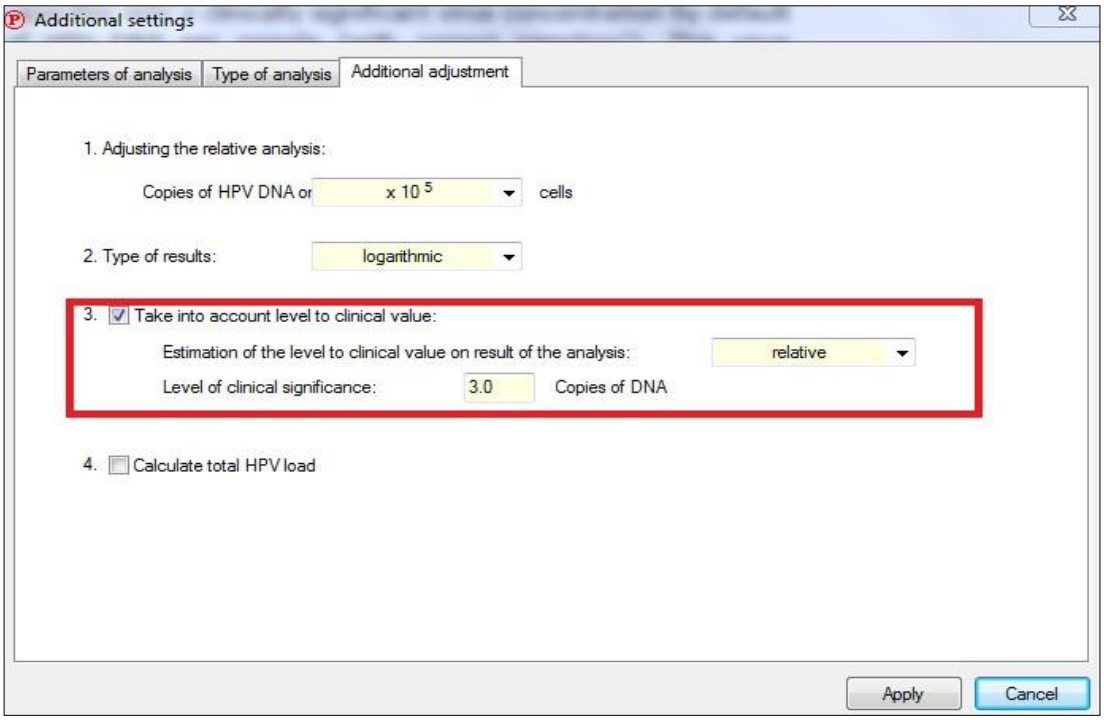
Date
Signature

In the samples containing HPV DNA (specific product), the absolute quantity of this virus type (the degree of concentration common logarithm, number of copies of the HPV DNA per sample) will be specified in the line with the name of this type of HPV in the “Quantitative” field (absolute analysis). In this case, the amplification result of the internal control is not taken into account.

The SIC value must be considered when analyzing results: SIC values lower than 4.0 should be considered as an insufficient amount of sample, and the sampling procedure must be repeated.

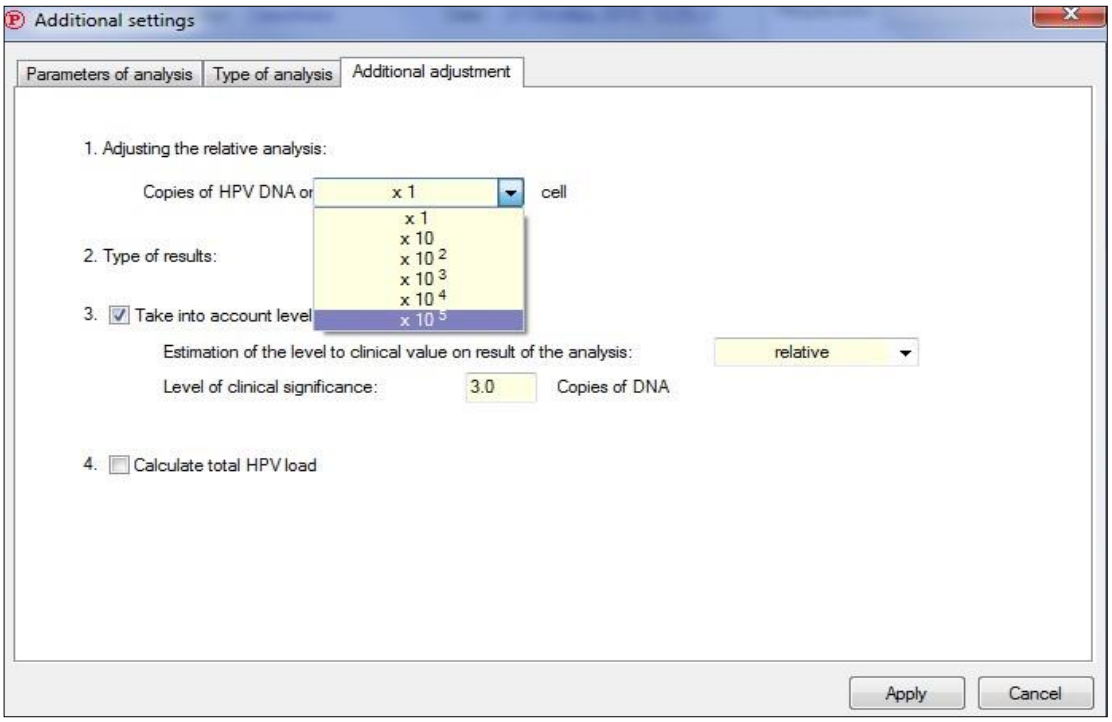
In the samples free of HPV DNA, the program registers a negative result for the specific product and positive result for the internal control.

The software specifies only a clinically significant virus concentration by default (more than 10^3 copies of HPV DNA per 10^5 human cells (with correct sampling)). This virus concentration characterizes a high infection rate, which can lead to the development of cervical neoplasia. For samples with lower concentration the result is registered in the format “< [the default amount or amount determined by user]”. The software restriction of the virus concentration can be removed by the user or replaced with another value and type of analysis.



The amount of HPV DNA indicated in the “Relative” field will be normalized to the number of human cells in the test sample. By default, normalizing is performed to 10^5 human cells.

If needed, the user can change the normalization parameter. To do this, click the “Change parameters of data analysis” button, in the opened window click the “Additional settings" button, then select the "Additional adjustment” tab.



The data obtained with relative type of analysis allows one to monitor the dynamics of the viral load changes during treatment, and also to carry out a comparative analysis of the different samples.

The result of the total HPV load calculating for each sample is presented in a specialized report.

In the "Qualitative" field (qualitative analysis), if it is carried out, only the presence or absence of HPV DNA in the sample is indicated.

In the absence of specific HPV signal and IC signal the program registers unreliable result. It is necessary to repeat the analysis for the given sample. An unreliable result may be due to the presence of inhibitors in the DNA preparation; incorrect implementation of the analysis protocol, violation of the amplification temperature regime, etc. In this case, it is necessary to repeat PCR amplification, or DNA isolation and PCR, or sampling procedure for the given patient (performed sequentially).

For positive control samples, the program registers a positive result. When negative results are obtained, all results of corresponding experiment should be considered as false. All samples must be reanalyzed.

For negative control samples, the program registers a negative result. When positive results are obtained, all results of the corresponding experiment should be considered as false and the PCR laboratory must be decontaminated.

11. SPECIFICATIONS

a. The analytical specificity of the **HPV-QUANT-21® quantitative 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 HPV DNA are to be registered positive for specific product (a fragment of the HPV genome). The samples free of HPV DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum*, *Ureaplasma parvum, Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Candida albicans*, *Streptococcus sp*., *Staphylococcus sp*., *Lactobacillus spp*., EBV, HHV6, HHV8, HSV1, HSV2, VZV.

b. In a determination of analytical sensitivity, the **HPV-QUANT-21® quantitative PCR Detection Kit** demonstrated the ability to reproducibly detect 5 or more copies of purified pathogens DNA per PCR reaction (10^3 copies/mL DNA sample). The HPV copies' number was determined by Poisson analysis.

The **HPV-QUANT-21® quantitative PCR Detection Kit** detects one CFU of the pathogen per PCR reaction. This analytical sensitivity was determined by serially diluting pathogens infected cultures in culture transport media. Samples of each dilution were processed and tested by the standard Kit procedure. Each of the replicates containing 1 CFU per amplification reaction gave a strong positive signal.

The analytical sensitivity depends on the type of biomaterial, DNA extraction kit and the final volume of extracted DNA elution. For example: the analytical sensitivity of the kit is 600 copies/sample when DNA is extracted from a sample by **PREP-NA PLUS**, **PREP-GS PLUS** and **PREP-MB RAPID** extraction kits (elution volume is 300 µL).

c. Sample Intake Control

During amplification of biological samples containing human genomic DNA the Real-Time PCR instrument should record the exponential growth of the fluorescence level in the corresponding tube. SIC values lower than 4.0 should be considered as an insufficient amount of sample, and the sampling procedure must be repeated.

During amplification of biological samples that do not contain the human genomic DNA the Real-Time PCR instrument should record the absence of exponential growth of the fluorescence level in the corresponding tube.

d. Diagnostic characteristics

Number of samples (n) - 191;

Diagnostic sensitivity (95% CI) – 99.3% (96.7-100%);

Diagnostic specificity (95% CI) – 99.9% (99.8-99.9%).

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA PLUS** REF P-002/2EU, **PREP-GS PLUS** REF P-003/2EU and **PREP-MB RAPID** REF P-116-A/8EU extraction kits.

12. TROUBLESHOOTING

Table 4. 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
SIC	-	PCR inhibition Insufficient amount of DNA	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

The quality control procedures 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 **HPV-QUANT-21® quantitative PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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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
	Caution	VER	Version
NON STERILE	Non-sterile	CONTROL +	Positive control
	Do not reuse		

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