CE IVD

For professional use only

HPV-QUANT-21® quantitative REAL-TIME PCR Detection Kit

USER MANUAL

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC

Russia, 142281, Moscow Region,

Protvino, 20 Zheleznodorozhnaya Street,

Phone/fax:

+7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.ru

Customer service department E-mail: hotline@dna-technology.ru

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1. INTENDED USE

The ***HPV-QUANT-21® quantitative*** **REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The ***HPV-QUANT-21® quantitative*** **REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The ***HPV-QUANT-21® quantitative*** **REAL-TIME 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 scrapes, prostate fluid, ejaculate, urine, biopsy material.

The ***HPV-QUANT-21® quantitative*** **REAL-TIME 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 regard to their oncogenic properties human papillomaviruses 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 The ***HPV QUANT-21® quantitative*** **REAL-TIME PCR Detection Kit.**

The ***HPV QUANT-21® quantitative*** **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 user manual.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. DNA molecules are heat denatured while the cyclic amplification program proceeds. Target-specific primers bind to the denatured DNA templates in the presence of dNTP's, and Taq-polymerase. Taq-polymerase extends the primers thus providing the synthesis of complementary DNA chains and amplification of target DNA sequence.

To increase the specificity and sensitivity of reaction, a paraffin layer separates the PCR-mix and Taq-polymerase. It hampers the admixture of PCR components at low temperatures, thus providing a "hot-start" feature, which prevents unspecific PCR.

The ***HPV-QUANT-21® quantitative*** **REAL-TIME PCR Detection Kit** is based on measurement of fluorescence at every cycle of reaction. The PCR-mix contains target-specific hydrolyzing probes bearing reporter and quencher molecules. While the probe is intact, these molecules are close enough to provide effective quenching. Once hybridized to a target sequence, the probe is hydrolyzed by Taq-polymerase. Thereby reporter and quencher become separated and fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is analyzed with a Real-time PCR instrument data collection unit and the software provided.

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

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 fluorescent dyes are assigned to individual types of sequences. The Fam, Rox and Cy5 dyes label are used to detect specific sequences. The Hex dye label is used to detect IC. The Fam dye label is also used for SIC detection. The SIC allows to exclude preanalytical error. Use of several distinguishable dyes allows detection of several PCR products simultaneously in one tube.

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				Colour of the PCRmix
	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 (Ct, 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: **DT*lite*** or **DT*prime* REAL-TIME Thermal Cyclers** for ***HPV-QUANT-21® quantitative* REAL-TIME PCR Detection Kit** (see the catalogue at www.dna-technology.ru/en to see available supply options).

The current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

3. CONTENT

The ***HPV-QUANT-21® quantitative* REAL-TIME PCR Detection Kit** content is represented in Table 2.

Table 2. The HPV-QUANT-21® quantitative REAL-TIME 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 per tube)	24 8-tube strips
MAX Taq-polymerase solution	Colorless transparent liquid	2000 µL (500 µL per tube)	4 tubes
Mineral oil	Colorless transparent viscous oily liquid	4 mL (1,0 mL per tube)	4 tubes
Positive control	Colorless transparent liquid	160 µL	1 tube

The kit is intended for single use and designed for 24 tests.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts;
- Place swabs into 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;
- Nucleic acid extraction kit (PREP-NA-PLUS (REF P-002/2EU), PREP-GS-PLUS (REF P-003/2EU) and PREP-MP Extraction Kits are recommended);
- High speed centrifuge (RCF 16000 x g);
- Microcentrifuge with rotor for strips;
- Solid-state thermostat (temperature range 50-65 °C);
- PCR tube rack for 1.5 mL tubes;
- 1.5 mL PCR tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips;
- Single channel pipettes (volume range 1.0-20 µL, 1.0-200 µL, 100-1000 µL);
- RNase and DNase free filtered pipette tips (volume range 20 µL, 200 µL, 1000 µL);

- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for strips;
- Microcentrifuge with rotor for strips;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes in strips;
- Single channel pipettes (volume range 0.5-10 µL, 5-40 µL, 40-200 µL, 100-1000 µL);
- RNase and DNase free filtered pipette tips (volume range 20 µL, 200 µL, 1000 µ);
- 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 http://www.dna-technology.ru/eng/support/.

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

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

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

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

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

If you face to any undescribed issues contact our representative in EU or customer service department regarding quality issues with the kit:

Technical support E-mail: hotline@dna-technology.ru, www.dna-technology.ru.

6. WARNINGS AND PRECAUTIONS

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 besterile, 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. Use 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 121ºC before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, 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. 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 Master 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 breach;
- 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*** **REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from the epithelial scrapes, prostate fluid, ejaculate, urine, biopsy 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 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.

Scrapes sampling

The scrapes sampling is held by sterile swab to 1.5 mL plastic tubes containing transport medium designed by the manufacturer for transportation and storage biomaterial samples for PCR.

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.

Material

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 must not perform hygiene procedures or syringing prior the sampling procedure.

To interpret results successfully and robustly, sample must contain the largest possible number of epithelial cells with minimum amounts of mucus and blood. Inappropriate sampling may result in doubtful results and sample collection may need to be repeated.

The features of urethral sampling

The sampling is held by sterile disposable swab.

Patient must not urinate within 1.5-2 hours prior to sampling procedure.

The external urethral orifice must be treated with a swab moistened with sterile physiological saline solution just prior to the sampling procedure.

Carefully insert the swab into the urethra to a depth of 1-1.5 cm, then with careful rotational movements, move it to the external urethral orifice.

The features of the vaginal 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.

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 cervical sampling

Remove mucus with a swab prior to sampling, and treat the cervix uteri with sterile physiological saline solution.

Carefully insert sampling swab into the cervix to a depth of 0.5-1.5 cm.

Avoid contact with vaginal wall when removing the swab.

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.

Guidelines for taking sample to transport medium:

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.

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.

 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.

The first portion of morning urine

The first portion of morning urine in the amount of not less than 20 – 30mL 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.

Biopsy samples

The biopsy samples (bioptat) are placed into a sterile tube with sterile physiological saline solution or water (no more than 5mL) or into an empty sterile tube.

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.

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.

Preparation of (clinical) material for the assay

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

- 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.
- Vortex the tube (15 seconds).
- Remove the device tip from tube and throw out.
- Transfer into 1,5 mL clean tube.
- Centrifuge the tube at 16000 x g for 10 min.
- Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

Preparation of the urine material:

- Transfer 1,0 mL of the material from container into 1,5 mL plastic tube.
- Centrifuge the tube at 16000 x g for 10 minutes.
- Remove the supernatant completely.
- Add 1,0 mL of the sterile saline to the precipitate.
  Centrifuge the tube at 16000 x g for 10 minutes.
- Remove supernatant leaving approximately 100 µL (precipitate + liquid fraction) in the tube.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in PREP-NA-PLUS, PREP-GS-PLUS and PREP-MP 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-MP Extraction Kits are recommended.

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.

Assay procedure:

1. Mark the required number of 8-tube strips with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-).

One strip contain PCR-mixes for two 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.

2. Vortex the MAX Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.
3. Add 10 µL of MAX Taq-polymerase solution into each tube. Avoid paraffin layer break.
4. Add one drop (~20 µL) of mineral oil into each tube of the strip. Close strips tightly.
5. Vortex tubes with samples, positive control and negative control for 3-5 seconds, then spin for 1-3 seconds.

⚠ Open 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 one to prevent contamination. Use filter tips.

6. Add 5 µL of DNA sample into corresponding strip. Do not add DNA into the "C-", "C+" tubes. Avoid paraffin layer break. Close the tubes tightly.
7. Add 5 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" strip and 5 µL of positive control (C+) into corresponding strip. Avoid paraffin layer break. Close the tubes tightly.
8. Spin strips for 3-5 seconds.
9. Set the strips into the Thermal Cycler. Try to place strips in the center of thermoblock.
10. Launch the RealTime_PCR application in "Device operation" mode. Upload the .ini file supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler's user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID's of the samples, specify the position of the strips in the thermal unit (p. 9) and run PCR. See table 3.

⚠ Amplification products can be stored at temperatures from 2 °C to 8 °C for one month or at temperatures from minus 20 °C for 12 months.

Table 3. The PCR program for DT*lite* and DT*prime* 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

9. CONTROLS

***HPV-QUANT-21® quantitative* REAL-TIME 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). SIC is needed for sample quality assessment. 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 with its volume according DNA extraction kit used.

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 absence 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 are absent.

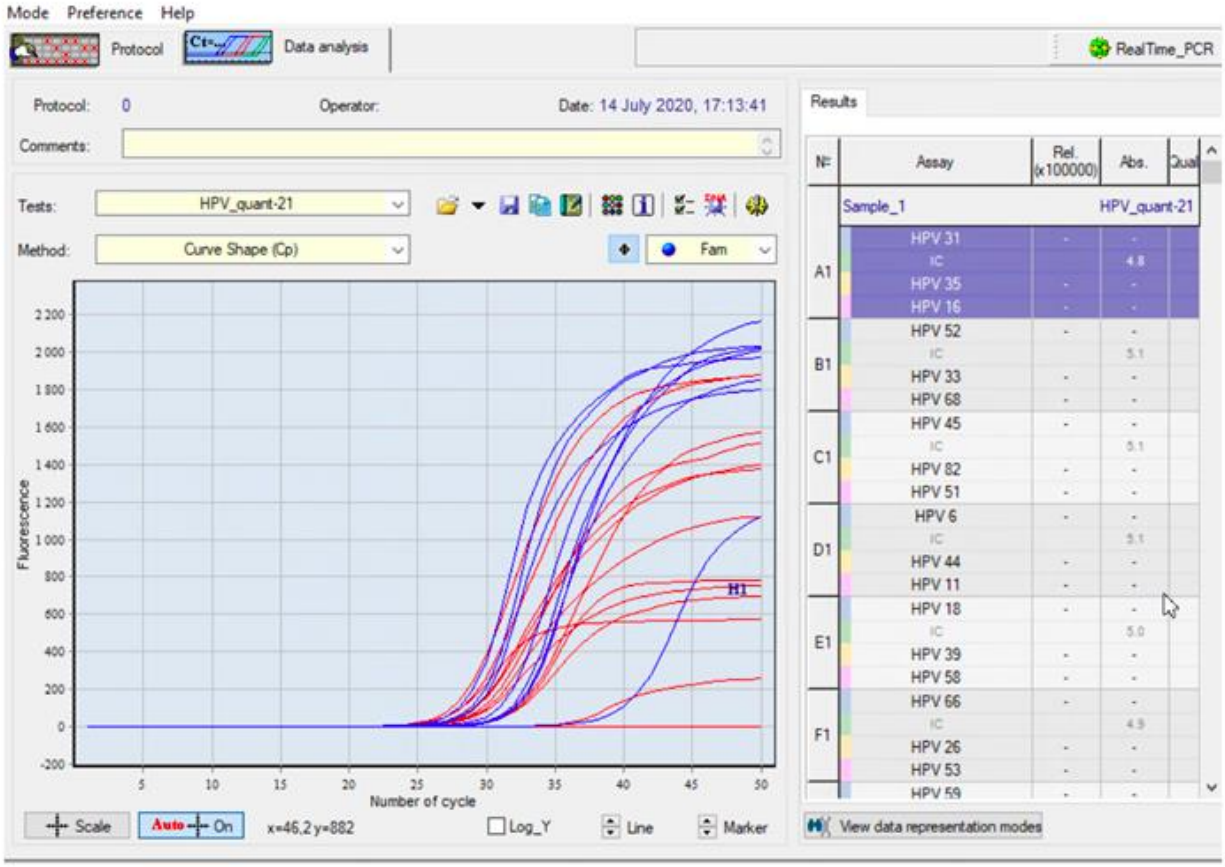
If positive control (C+) has **not** positive result for the specific product, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

If negative control (C-) has positive result for the specific product, whole tests of current batch considered false. Decontamination is required.

10. DATA ANALYSIS

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

The Real-time PCR Thermal Cyclers detects and interprets results automatically. 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.


Mode Preference Help
Protocol
Data analysis
RealTime_PCR
Protocol: 0
Operator:
Date: 14 July 2020, 17:13:41
Comments:
Tests: HPV_quant-21
Method: Curve Shape (Cp)
Fam
Fluorescence
Number of cycle
H1
Scale
Auto On
x=46,2 y=882
Log_Y
Line
Marker
Results
№
Assay
Rel. (x100000)
Abs.
Sample_1
HPV_quant-21
A1
HPV 31
IC
HPV 35
HPV 16
B1
HPV 52
HPV 33
HPV 68
C1
HPV 45
HPV 82
HPV 51
D1
HPV 6
HPV 44
HPV 11
E1
HPV 18
HPV 39
HPV 58
F1
HPV 66
HPV 26
HPV 53
HPV 59
View data representation modes

HPV

HPV_quant-21

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

Logotype

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.

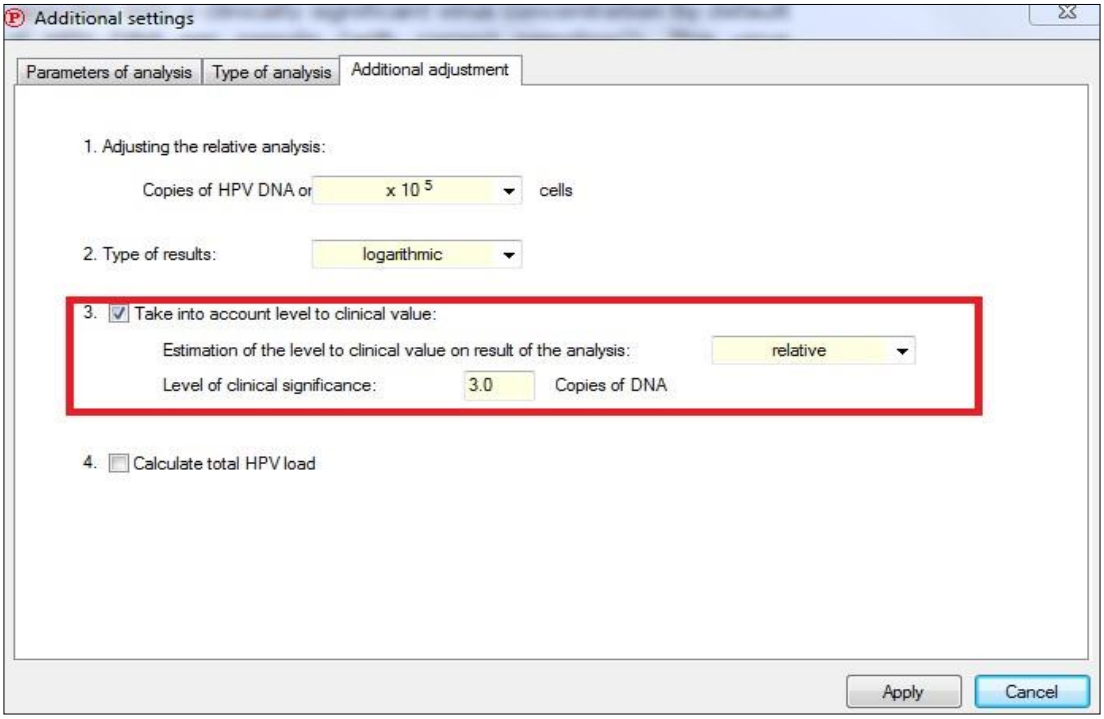
The SIC value must be considered when analyzing results: SIC values lower than 4.0 should be considered as an insufficient amount of sample, and the sampling procedure must be repeated.

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

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).

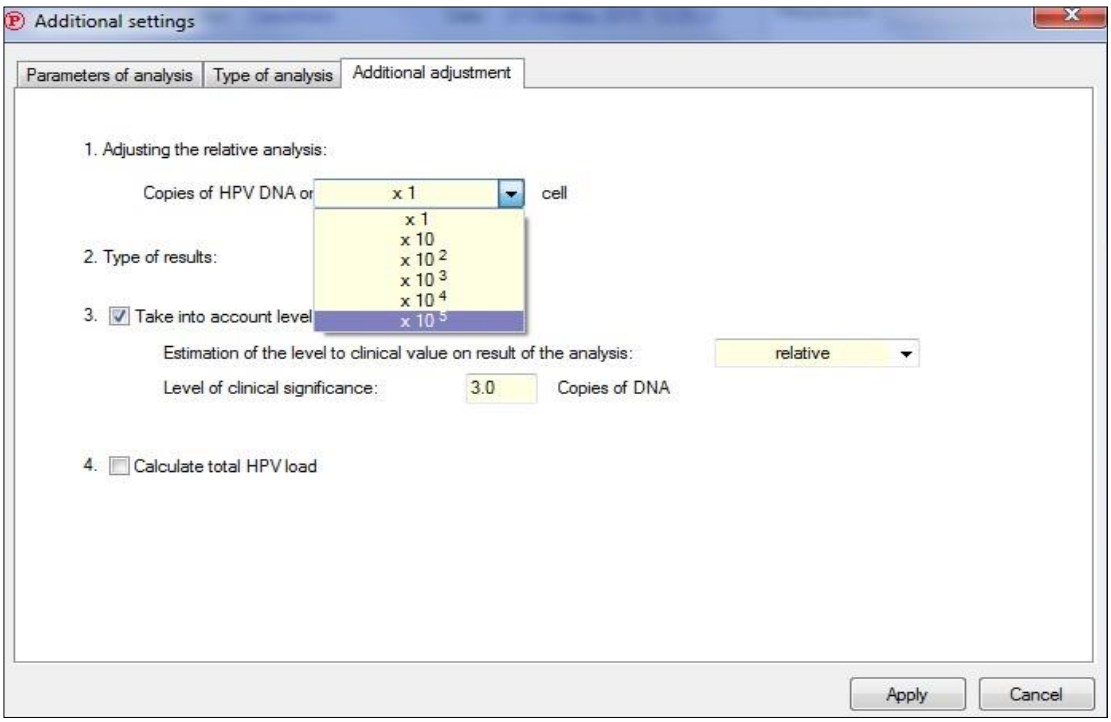
The software specifies only a clinically significant virus concentration by default (more than 10^3 copies of HPV DNA per sample (with correct sampling)). This virus concentration characterizes

a high infection rate, which can lead to the development of cervical neoplasia. For samples with a lower concentration, the program registers a negative result. 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, normalising 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*** **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 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*** **REAL-TIME 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*** **REAL-TIME PCR Detection Kit** detect 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-MP 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-002/1EU, PREP-GS-PLUS REF P-003/1EU and PREP-MP 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

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

Technical support E-mail: hotline@dna-technology.ru, www.dna-technology.ru.

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 ISO 9001:2015 and ISO 13485:2016:

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

Contact our customer service with quality issues of ***HPV-QUANT-21® quantitative*** **REAL-TIME PCR Detection Kit**:

Technical support E-mail: hotline@dna-technology.ru

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

Russia, 142281, Moscow Region,

Protvino, 20 Zheleznodorozhnaya Street,

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.ru

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels,

Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

14. KEY TO SYMBOLS

IVD	In vitro diagnostic medical device		Date of manufacture
	Temperature limitation		Consult instructions for use
Σ	Sufficient for	REF	Catalogue number
	Use by		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
CONTROL −	Negative control	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Do not reuse
NON STERILE	Non-sterile		