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**For professional use only**

HPV QUANT Quantitative Real-Time PCR Kit USER MANUAL

Available supply options:

HPV QUANT-21, **REF** R1-P317-S3/5EU

HPV QUANT-15, **REF** R1-P316-S3/4EU

HPV QUANT-4, **REF** R1-P315-S3/4EU

Read entire protocol before use

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1. INTENDED USE

- 1.1.** The DNA-Technology **HPV QUANT Quantitative Real-time PCR Kit** is intended for the specific identification, typing and quantification of low-risk (HPV 6, 11, 44) and high-risk (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 papillomavirus DNA.
- 1.2.** The kit is intended for research and diagnostic applications.

2. KIT CHARACTERISTICS

2.1. Application

Cervical cancer is one of the main malignant diseases of the reproductive system. This type of tumor is next in the prevalence rate (after breast cancer), and makes up about 14% of all malignant tumors in women. There is a high level of advanced stages of cervical cancer in Russia- more than 40% of all cases detected. More than 6,000 women die annually from cervical cancer in Russia. In the age group of 16-40 years, cervical cancer is the second leading cause of mortality of patients with malignant neoplasms after breast cancer.

Papillomavirus infection is now a well-established etiologic agent of cervical cancer. It is shown that the DNA of human papillomavirus (HPV) is detected in more than 99.5% of all biopsies in patients with cervical cancer.

About 30 from more than 120 currently known types of HPV can infect the epithelium of the urogenital tract. By the ability to cause canceration of the epithelium, they are figuratively divided into 2 groups - high and low oncogenic risk.

The HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82 belong to the group of high oncogenic risk. Type 16 is the most common in Europe and is detected in more than 50% of all cervical cancers.

The HPV types 6, 11, 36, 42, 43, 44, 46, 47, 50 belong to the group of low oncogenic risk. Infection caused by these types usually proceeds as benign lesions of the cervix and larynx condylomata.

HPV usually infects the epithelium of the cervical transformation zone, and causes precancerous changes. It is considered that the progress from epithelium infection to the cervical cancer development takes at least 5 years (an average of 10-20 years), and only in very rare cases this process can occur in 1-2 years. Therefore, tests allowing to detect the presence of HPV infection and precancerous changes or cancer in the early stages of the disease should hold a high position in the anticancer prevention measures.

Cytological screening is the most widely applied method for diagnostic of cervical cancer and precancerous epithelial changes. But accuracy of this diagnosis method varies greatly depending on the technique of sampling, smears fixing and preparing, qualifications of the researcher.

The development of molecular biology and the establishment of the HPV role in the cervical cancer development have led to the use of DNA diagnosis of HPV infection as the basis for screening and preventing this serious disease. Many countries accumulated a positive experience in application of DNA tests for determining HPV DNA in screening studies.

Real-time PCR is the best method for HPV DNA-diagnosis, which allows to quickly perform a study and obtain a result. In addition, real-time PCR allows not only screening, but also a quantitative study of the viral load.

2.2. 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.

Real-time PCR technology 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 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 (see tables 1, 2, 3 for the reference). The FAM dye label is also used for SIC detection. Use of several distinguishable dyes allows detection of several PCR products simultaneously in one tube.

Defined tubes in the HPV QUANT-15 and HPV QUANT-21 format contain additional probe with ROX dye label – "Marker" (see tables 1, 2, 3 for the reference). 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.

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.

HPV QUANT supplies options, strips content, fluorescent chemistry/detection channels and color tags are shown in the Tables 1, 2, 3.

Table 1. HPV QUANT-4

PCR-mix	Detection channel				Buffer color	Paraffin color
	Fam	Hex	Rox	Cy5		
HPV 6, 11	HPV 6 type	IC	-	HPV 11 type	Blue	White
HPV 16, 18	HPV 18 type	IC	-	HPV 16 type	Colorless	Blue
SIC	SIC	IC	-	-	Colorless	White

Table 2. HPV QUANT-15

No of the tube	Detection channel				Buffer color
	Fam	Hex	Rox	Cy5	
1,5	HPV 16, 31, 33, 35, 52, 58	IC	-	HPV 56	Blue
2,6	HPV 18, 39, 45, 59	IC	-	HPV 6, 11	
3,7	HPV 51	IC	Marker	HPV 68	
4,8	SIC	IC	-	-	

Table 3. HPV QUANT-21

No of the tube	Detection channel				Buffer color
	Fam	Hex	Rox	Cy5	
1	HPV 31 type	IC	HPV 35 type	HPV 16 type	Colorless
2	HPV 52 type	IC	HPV 33 type	HPV 68 type	
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	-	

2.3. KIT CONTENTS

The Kit is available in three supply options depending on the number of HPV types detected:

- HPV QUANT-4 is intended for HPV 6, 11, 16, 18 types detection;
- HPV QUANT-15 is intended for HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 types detection;
- HPV QUANT-21 is intended for HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 detection.

The kit includes:

1. HPV QUANT-4 (48 tests)

- Paraffin sealed PCR-mix for HPV 6, 11 types amplification - 6 8-tubes strips (20 µL in each tube);
- Paraffin sealed PCR-mix for HPV 16, 18 types amplification - 6 8-tubes strips (20 µL in each tube);

- Paraffin sealed PCR-mix for Sample intake control (SIC) amplification - 6 8-tubes strips (20 µL in each tube);
- Taq-polymerase solution – 3 tubes (500 µL in each tube);
- Mineral oil – 3 tubes (1 mL in each tube);
- Positive control sample HPV QUANT-4 – 1 tube (150 µL);
- Cap strips – 18 8-cap strips.

2. HPV QUANT-15 (48 tests)

- Paraffin sealed PCR-mix - 24 8-tubes strips (20 µL in each tube);
- MAX Taq-polymerase solution – 4 tubes (500 µL in each tube);
- Mineral oil – 4 tubes (1 mL in each tube);
- Positive control sample HPV QUANT-15 – 1 tube (150 µL);
- Cap strips – 24 8-cap strips.

3. HPV QUANT-21 (24 tests)

- Paraffin sealed PCR-mix - 24 8-tubes strips (20 µL in each tube);
- MAX Taq-polymerase solution – 4 tubes (500 µL in each tube);
- Mineral oil – 4 tubes (1 mL in each tube);
- Positive control sample HPV QUANT-21 – 1 tube (150 µL);
- Cap strips – 24 8-cap strips.

2.4. Analysis time – from 2.5 hours (including sample preparation).

3. KIT SPECIFICATIONS

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). The normalization is performed to one cell by default. The normalization parameter (from one to 10⁵ cells) can be changed by the user. At the user request, it is possible to calculate the total HPV load (9.4). Qualitative analysis implies only the HPV DNA detection in the sample.

3.1. Specificity

The lists of HPV types identified by the kit are presented in Tables 1, 2 and 3.

During amplification of biological samples containing DNA of the detectable HPV type the Real-Time PCR instrument should record the exponential growth of the fluorescence level in the corresponding tube.

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

3.2. 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.

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.

4. WARNING AND PRECAUTIONS

As part of industrial and personal hygiene and general safety practices, avoid all unnecessary exposure to the chemical components of this kit and ensure prompt removal from skin, eyes, and clothing upon contact.

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

4.1. General warnings and precautions

Handle and dispose all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Handle and dispose all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid producing spills or aerosol. Waste must be handled and disposed according to adequate safety measures. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the regulations in force.

Read the **User manual** provided with the kit before running the assay.

While running the assay follow the instructions listed in the **User manual**.

Do not use the kit after the expiry date provided.

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.

4.2. Warnings and precautions for molecular biology

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.

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.

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.

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.

4.3. 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.

5. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following instruments and materials are required when using HPV QUANT Quantitative Real-time PCR Kit:

- PCR cabinet;
- Real-time PCR thermal cycler DTlite¹, DTprime², DT-96 (see the catalogue at www.dna-technology.ru/eng/ to see available supply options); software version 7.3.5.84 or higher. The current version of the software is available for download at <http://www.dna-technology.ru/eng/support/>; HPV_QUANT_en.ini file).
- High-speed centrifuge (16 000 g);
- Microcentrifuge with rotor for strips;
- Refrigerator with a freezer chamber;
- Solid-state thermostat (temperature range 50-65 °C);
- PCR tube rack for 1.5 ml tubes;
- PCR tube rack for strips of eight 0.2 ml tubes;
- 1.5 ml PCR tubes;
- Single channel pipettes (volume range 2.0-20 µl, 20-200 µl, 100-1000 µl);
- RNase and DNase free filtered pipette tips (volume range 20 µl, 200 µl, 1000 µl);
- Powder-free surgical gloves;
- Container for used pipette tips;
- Disinfectant solution;
- "DNA-Technology" made transport medium (**REF** P-001/1EU);
- Nucleic acid extraction kit ("DNA-Technology" PREP-NA PLUS DNA/RNA Extraction Kit (**REF** P-002/2EU) or PREP-GS PLUS DNA Extraction Kit (**REF** P-003/2EU) are recommended).

Software: The recommended version of software is 7.7.5.44, software older than version 7.3.3.10 is not recommended; "HPV_QUANT_en.ini" file. The recommended version can be

¹ - supported by 4S1; 4S2; 5S1; 5S2; 6S1; 6S2 instruments.

² - supported by 4M1; 4M3; 4M6; 5M1; 5M3; 5M6; 6M1; 6M3; 6M6 instruments.

changed due to software update. The recent version of DT-96 software is available for download at <http://www.dna-technology.ru/eng/support/>.

6. SAMPLES

Epithelial scrapes from cervical canal, cervix uteri and urethra can be used for analysis depending on professional prescription.

6.1. Urogenital scrapes sampling

The urogenital sampling is held by sterile swab (or brush). The sample is then transferred to 1.5 mL plastic tubes containing DNA-Technology transport medium (**REF** P-001/1EU).

6.1.1 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.

6.1.2 Material

Professional prescription is required to localize the place of sampling (urethra, cervix or vaginal wall). 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.

6.2. Features of urethral sampling

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.

In the case of purulent discharge, the sample must be taken 15-20 minutes after urinating.

Carefully insert the swab into the woman's urethra to a depth of 1-1.5 cm. A child's sample must be taken from the external urethral orifice.

6.3. Features of cervical sampling

Remove mucus with a swab prior to sampling, and treat the cervix 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.

6.4. 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.

6.5. Guidelines for taking sample to transport medium

- 6.5.1. Open the tube
- 6.5.2. Scrape epithelial cells from the corresponding biotope (i.e. urethra, cervix uteri, cervical canal) with a sterile swab.
- 6.5.3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab. Use a new swab if you need to repeat sampling or to take sample from another biotope.
- 6.5.4. Close the tube tightly and mark it.

6.6. Samples shipping and storage



Overall time from the sample intake until analysis must not exceed 24 hours.

Store samples at the temperatures between 2 °C and 8 °C.

If sample cannot be delivered to laboratory within 24 hours, it can be frozen once.

7. PROCEDURE

7.1. DNA extraction



A DNA extraction kit is not included in **HPV QUANT Quantitative Real-time PCR Kit**.

7.1.1. DNA extraction from urogenital samples using PREP-NA PLUS DNA/RNA Extraction Kit (REF P-002/2EU)



If precipitate occurs in the "Lysis buffer", dissolve it by heating solution up to 65 °C for 10 minutes prior to use. Use RNase and DNase-free pipette tips only.

7.1.1.1. Centrifuge the tubes with samples at 13 000 rpm for 10 minutes.

7.1.1.2. Remove supernatant, leaving approximately 100 µL of liquid (precipitate+liquid fraction).



Perform each step of DNA extraction procedure for negative control ("C-") in parallel with test samples. Add 100 µL of transport medium in a new 1.5 ml tube and perform the procedure according to p. 7.1.1.3-7.1.1.18 of current manual.

7.1.1.3. Add 300 µl of Lysis buffer into each tube. Avoid contact of pipette tip with the tube edges.

7.1.1.4. Close the tubes tightly. Vortex the tubes for 3-5 seconds.

7.1.1.5. Incubate the tubes at 65 °C for 15 minutes. Centrifuge the tubes at 13 000 rpm for 30 seconds to drop down the condensate.

7.1.1.6. Add 400 µL of Precipitation buffer into the tubes and vortex the tubes for 3-5 seconds.

7.1.1.7. Centrifuge the tubes at 13 000 rpm for 15 minutes.

7.1.1.8. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).

7.1.1.9. Add 500 µl of Washout solution N°1 to the pellet and invert the tube 3-5 times gently.

7.1.1.10. Centrifuge the tubes at 13 000 rpm for 5 minutes.

7.1.1.11. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).

- 7.1.1.12. Add 300 µl of Washout solution N°2 to the pellet and invert the tube 3-5 times gently.
- 7.1.1.13. Centrifuge the tubes at 13 000 rpm for 5 minutes.
- 7.1.1.14. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).
- 7.1.1.15. Open the tubes and dry the pellet at 65 °C for 5 minutes.
- 7.1.1.16. Add 300 µl of Dilution buffer to the pellet. Centrifuge the tubes for 3-5 seconds to collect drops.
- 7.1.1.17. Incubate the tubes at 65 °C for 10 minutes. Vortex the tubes for 3-5 seconds.
- 7.1.1.18. Centrifuge the tubes for 30 seconds at 13 000 rpm to collect drops.
- DNA sample is ready for PCR performing.
- Store the DNA sample at minus 20 °C for one month or at minus 70 °C for one year.

7.1.2. DNA extraction from urogenital samples using PREP-GS PLUS DNA Extraction Kit (REF P-003/2EU)



If precipitates occur in the "Lysis buffer" or Washout solution N°1 dissolve it by heating solution up to 50 °C for 15-20 minutes prior to use. Use RNase and DNase-free pipette tips only.

- 7.1.2.1. Centrifuge the tubes with samples at 13 000 rpm for 10 minutes.
- 7.1.2.2. Remove supernatant, leaving approximately 50 µL of liquid (precipitate+liquid fraction).



Perform each step of DNA isolation procedure for negative control ("C-") in parallel with test samples. Add 100 µL of transport medium in new 1.5 ml tube and perform the procedure according to par. 7.1.2.3-7.1.2.21 of current manual.

- 7.1.2.3. Mix Lysis buffer with the Sorbent in a separate tube as follows:
- 150x(N+1) µl of Lysis buffer;
 - 20x(N+1) µl of previously resuspended Sorbent,
- (N+1)- number of samples to be analyzed including "C-" (N) and one reserve sample.
- 7.1.2.4. Add 170 µl of prepared mixture into each tube.
- 7.1.2.5. Close the tubes tightly, vortex the tubes for 3-5 seconds.
- 7.1.2.6. Incubate the tubes at 50 °C for 20 minutes.
- 7.1.2.7. Centrifuge the tubes at 13 000 rpm for 1 minute.
- 7.1.2.8. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).
- 7.1.2.9. Add 200 µl of Washout solution N°1 to the pellet. Vortex the tubes for 3-5 seconds.
- 7.1.2.10. Centrifuge the tubes at 13 000 rpm for 1 minute.
- 7.1.2.11. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).
- 7.1.2.12. Add 200 µl of Washout solution N°2 to the pellet. Vortex the tubes for 3-5 seconds.
- 7.1.2.13. Centrifuge the tubes at 13 000 rpm for 1 minute.
- 7.1.2.14. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).
- 7.1.2.15. Add 200 µl of Wash solution N°3 to the pellet. Vortex the tubes for 3-5 seconds.
- 7.1.2.16. Centrifuge the tubes at 13 000 rpm for 1 minute.

- 7.1.2.17. Remove supernatant completely. Avoid contact of pipette tip with pellet (use new tip for each tube).
- 7.1.2.18. Open the tubes and dry the pellet at 50 °C for 5 minutes.
- 7.1.2.19. Add 300 µl of Elution buffer to the pellet. Vortex the tubes for 5-10 seconds.
- 7.1.2.20. Incubate the tubes at 50 °C for 5 minutes.
- 7.1.2.21. Centrifuge the tubes at 13 000 rpm for 1 minute.
- Supernatant containing extracted DNA is ready for PCR performing.
- Store DNA samples at a temperature of 2 °C to 8 °C up to 7 days. Repeat steps according to p. 7.1.2.20-7.1.2.21 of current protocol prior to use of DNA sample in PCR.
- For more than 7 days, the DNA preparation should be stored at minus 20 °C (not more than 6 months).

7.2. Amplification

7.2.1. HPV QUANT-4:

Mark 1 tube from each type of strips (HPV 6,11; HPV 16,18 and SIC) with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-). 1 strip is intended for 8 samples analysis.

7.2.2. HPV QUANT-15:

Mark 4 tubes from strip with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-). 1 strip is intended for 2 samples analysis.

7.2.3. HPV QUANT-21:

Mark 1 strip with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-). 1 strip is intended for 1 sample analysis.

Example: for testing of 2 samples, mark 12 tubes in 3 different strips for HPV QUANT-4 format (6 for test samples, 3 for "C+", 3 for "C-"); 2 strips for HPV QUANT-15 format (1 strip for test samples and 1 for "C+" and "C-"); 4 strips for HPV QUANT-21 format (2 strips for test samples, 1 strip for "C-", 1 strip for "C+"). See Table 4 for reference.

Table 4. Example of tubes marking

	HPV QUANT -4	HPV QUANT-15	HPV QUANT -21
Sample 1	Tube 1 strip-1, strip-2, strip-3	Tubes 1-4	Tubes 1-8
Sample 2	Tube 2 strip-1, strip-2, strip-3	Tubes 5-8	Tubes 1-8
«C-»	Tube 3 strip-1, strip-2, strip-3	Tubes 1-4	Tubes 1-8
«C+»	Tube 4 strip-1, strip-2, strip-3	Tubes 5-8	Tubes 1-8

- 7.2.4. Mix the Taq-polymerase solution (MAX Taq-polymerase solution for HPV QUANT-15 and HPV QUANT-21) thoroughly (3-5 sec) then spin briefly (1-3 sec).
- 7.2.5. Add 10 µl of Taq-polymerase solution (MAX Taq-polymerase solution for HPV QUANT-15 and HPV QUANT-21) into each tube. Avoid paraffin layer break.
- 7.2.6. Add 20 µl of mineral oil (one drop approximately) into each tube. Close strips tightly.
- 7.2.7. Open the tube (strip), add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips.
- 7.2.8. Add 5.0 µl of DNA sample into each tube of a strip assigned to test samples. Do not add DNA samples into the tubes "C+" and "C-".
- 7.2.9. Add 5.0 µl of "C+" and "C-", which passed the DNA extraction procedure (see p. 7.1 for reference) into corresponding tubes.
- 7.2.10. Spin the strips briefly (1-3 sec.)
- 7.2.11. Set the strips to Real-time PCR instrument.

7.2.12. Launch "RealTime PCR" application in Device Handling mode. Upload the "HPV_QUANT_en.ini" file before the first run (see p. 7.3 for reference). Add "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" test in subsequent runs (see p. 7.4 for reference). Set the number of samples and specify tubes including "C+" and "C-". Specify position of strips in a thermoblock (see p. 7.4.6 for reference). Run PCR. Refer to tables 5 and 6 for the cycling programs corresponding to "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" tests. This program is displayed in a "Start run" window.



The amplification program for the "HPV_QUANT-15" differs from the amplification programs for the "HPV_QUANT-4" and "HPV_QUANT-21".

7.2.13. When selecting the tests "HPV_QUANT-4" and "HPV_QUANT-21" in the "Start run" window, the program shown in Table 5 should be displayed. If you select the "HPV_QUANT-15" test in the "Start Amplification Program" window, the program shown in Table 6 should be displayed.

Table 5. "HPV_QUANT-4" and "HPV_QUANT-21" cycling program for DTlite, DTprime and DT-96

Nº of block	Temperature, °C	min	sec	Number of cycles	Optical data collection mode	Type of block
1	80,0	0	30	1		Cycle
	94,0	1	30			
2	94,0	0	30	5		Cycle
	64,0	0	15		√	
3	94,0	0	10	45		Cycle
	64,0	0	15		√	
4	94,0	0	5	1		Cycle
5	10,0	Storage		Storage

Table 6. "HPV_QUANT-15" cycling program for DTlite, DTprime and DT-96

Nº of block	Temperature, °C	min	sec	Number of cycles	Optical data collection mode	Type of block
1	80,0	0	30	1		Cycle
	94,0	1	30			
2	94,0	0	30	5		Cycle
	60,0	0	15		√	
3	94,0	0	10	45		Cycle
	60,0	0	15		√	
4	94,0	0	5	1		Cycle
5	10,0	Storage		Storage

7.3 "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" test upload procedure. The following procedure is applicable for DTlite, DTprime and DT-96 Real-time PCR machines and must be held prior to **first PCR run on a given computer**

Software : version 7.9.5.15 or higher. The current version of the software is available for download at <http://www.dna-technology.ru/eng/support/>.

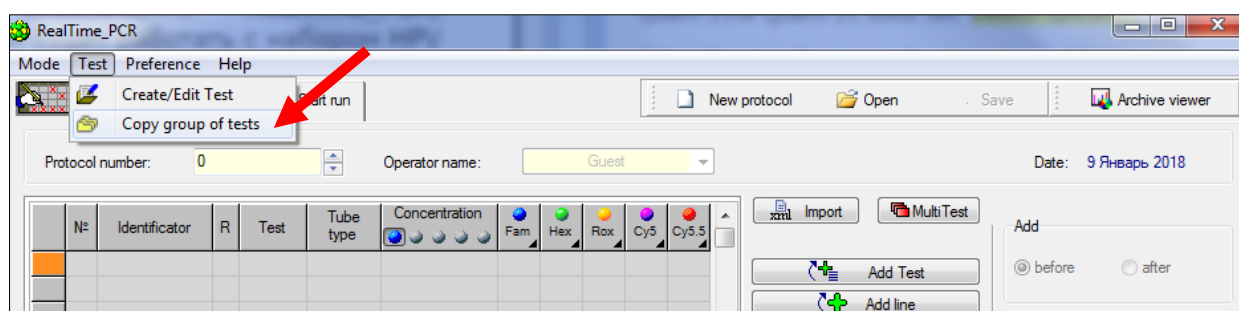


The screenshots are taken from 7.9.5.15 version.

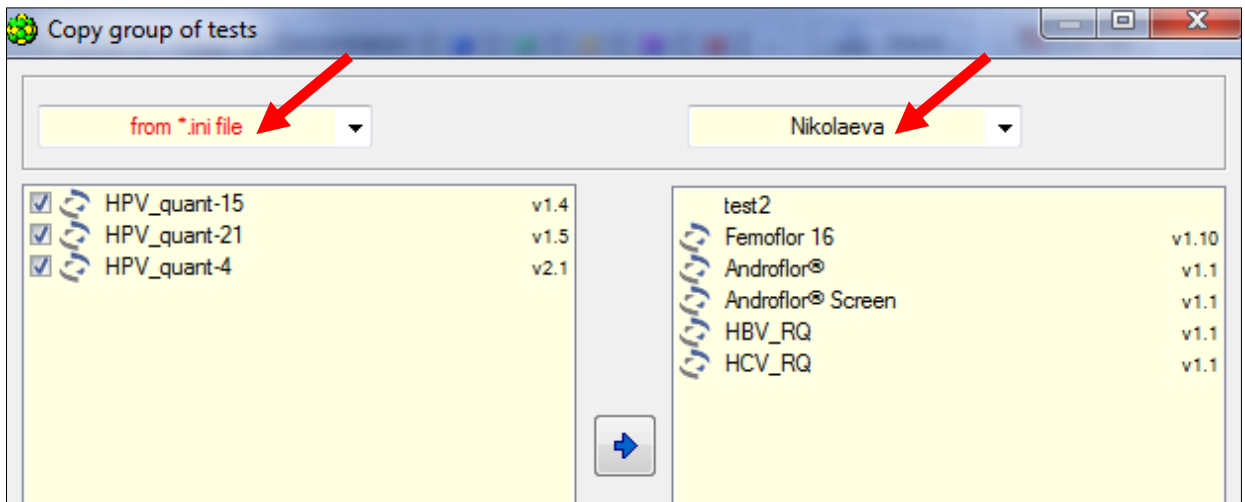
"HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" (HPV_QUANT_en.ini) for use with DTlite, DTprime and DT-96 Real-time PCR machines are provided by manufacturer. The uploading procedure must be held in a "Device operation" mode as follows:

7.3.1. Launch RealTime_PCR application. Choose operator to work with "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" tests. Set "Device operation" mode. Select directory for saving the data when adding new operator.


7.3.2. Select "Copy group of tests" bookmark in the "Test" menu.

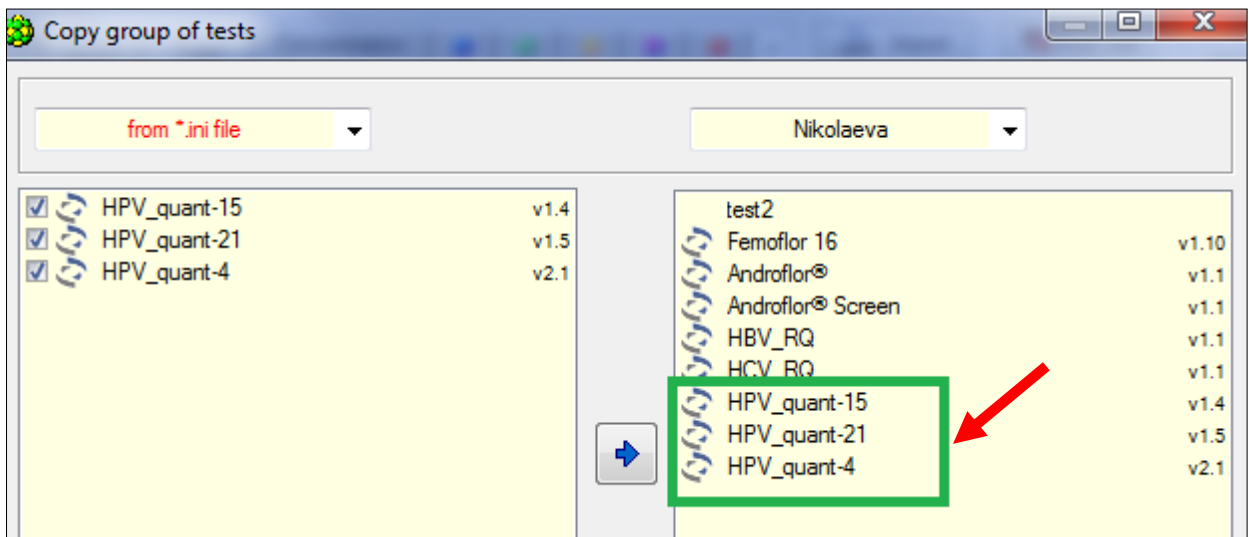


7.3.3. Select line "from *.ini file" in the left module of "Copy groups of tests" window. Open "HPV_QUANT_en.ini" file.



7.3.4. Select operator whose directory will be used to copy "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_quant-21" tests in a right module of "Copy group of tests" window.

7.3.5. Click  button. The selected test will appear in a right module of window. The operator for whom the test was copied can work now with "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" test.



Only the "Absolute" and "Relative" types of analysis are displayed in the settings of the "HPV_QUANT_en.ini" file by default.

Optionally, you can change the settings and add/remove the desired type of analysis, for example, add a "Qualitative" type of analysis.

To do this, after copying the test to the operator's directory, open the RealTime_PCR application in the Device operation mode, select the "Create/Edit test" tab in the "Test" menu, select the required test in the appeared window and click the "Edit current test" button.

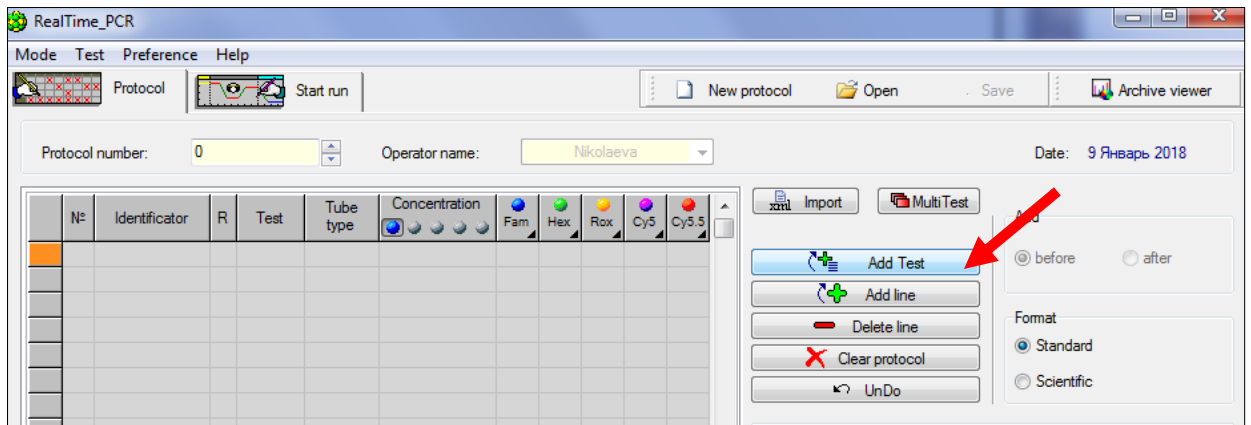
A "Test" window will appear in which you will need to click the "Additional settings" button, select the "Type of analysis" tab, specify the required type and click the "Apply" button.

The new settings will be saved and will be used in further work with the edited test.

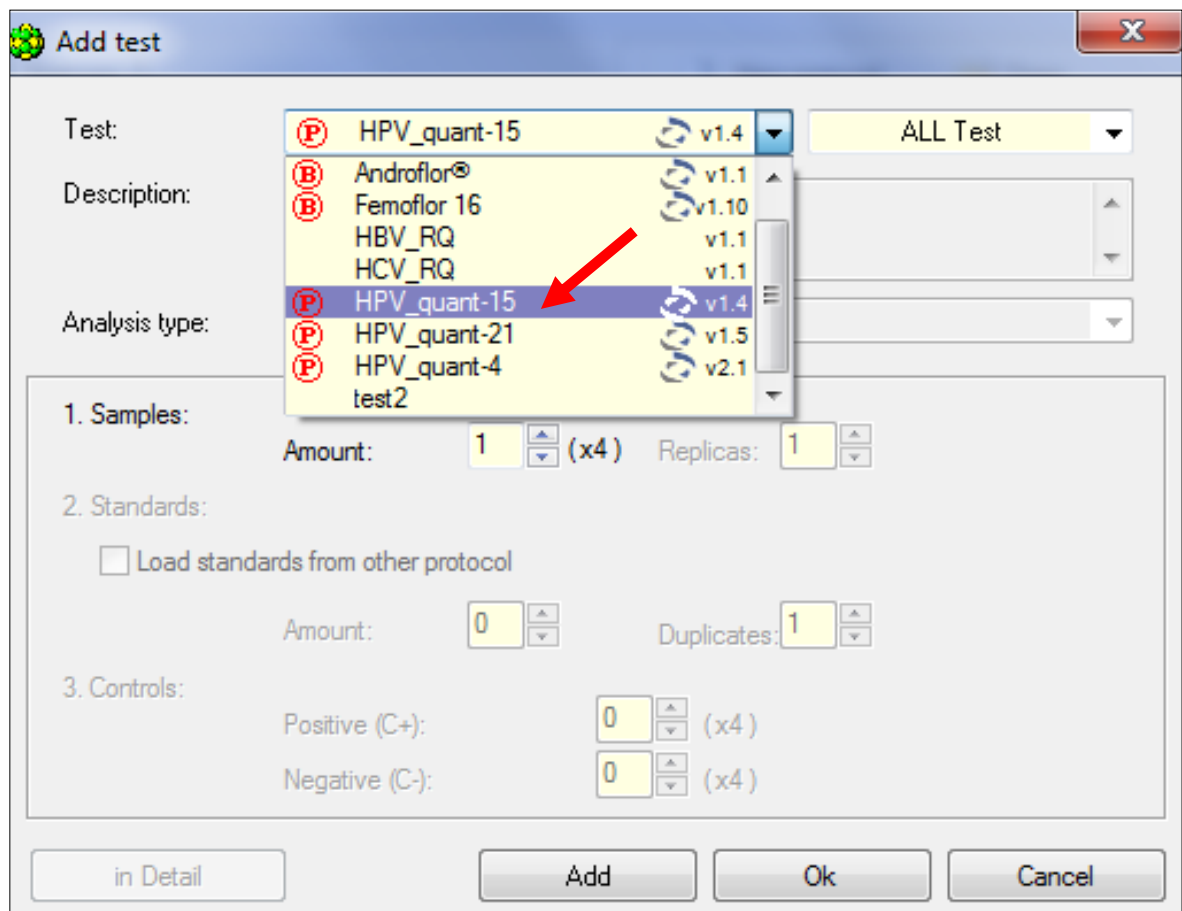
7.4. Routine work with "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" test

7.4.1. Launch the RealTime_PCR application. Choose operator for whom the test was copied (see p.7.3.4). Set "Device operation" mode.

7.4.2. Click "Add test" button.



7.4.3. Choose "HPV_QUANT-4", "HPV_QUANT-15" or "HPV_QUANT-21" test from the drop down menu.



7.4.4. Specify number of samples (C+ and C- should be identified as a test samples). Click "OK" button.

Add test

Test: HPV_quant-15 v1.4 ALL Test

Description: HPV-QUANT-15

Analysis type: HPV

1. Samples:
 Amount: (x4) Replicas:

2. Standards:
 Load standards from other protocol
 Amount: Duplicates:

3. Controls:
 Positive (C+): (x4)
 Negative (C-): (x4)

in Detail Add Ok Cancel

7.4.5. Specify the tubes.

Mode Test Preference Help

Protocol Start run

Protocol number: Operator name: Nikolaeva Date:

N°	Identifier	Test	Fam	Hex	Rox	Cy5
A1	1	Sample_1	HPV 16, 31, 33, 35, 52, 58	IC	-	HPV 56
B1	2		HPV 18, 39, 45, 59	IC	-	HPV 6, 11
C1	3		HPV 51	IC	Marker	HPV 68
D1	4		SIC	IC	-	-
E1	5	Sample_2	HPV 16, 31, 33, 35, 52, 58	IC	-	HPV 56
F1	6		HPV 18, 39, 45, 59	IC	-	HPV 6, 11
G1	7		HPV 51	IC	Marker	HPV 68
H1	8		SIC	IC	-	-
A2	9	Sample_3	HPV 16, 31, 33, 35, 52, 58	IC	-	HPV 56
B2	10		HPV 18, 39, 45, 59	IC	-	HPV 6, 11
C2	11		HPV 51	IC	Marker	HPV 68

Import MultiTest

Add Test Add line Delete line Clear protocol Undo

Add before after

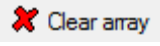
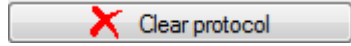
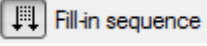
Format Standard Scientific

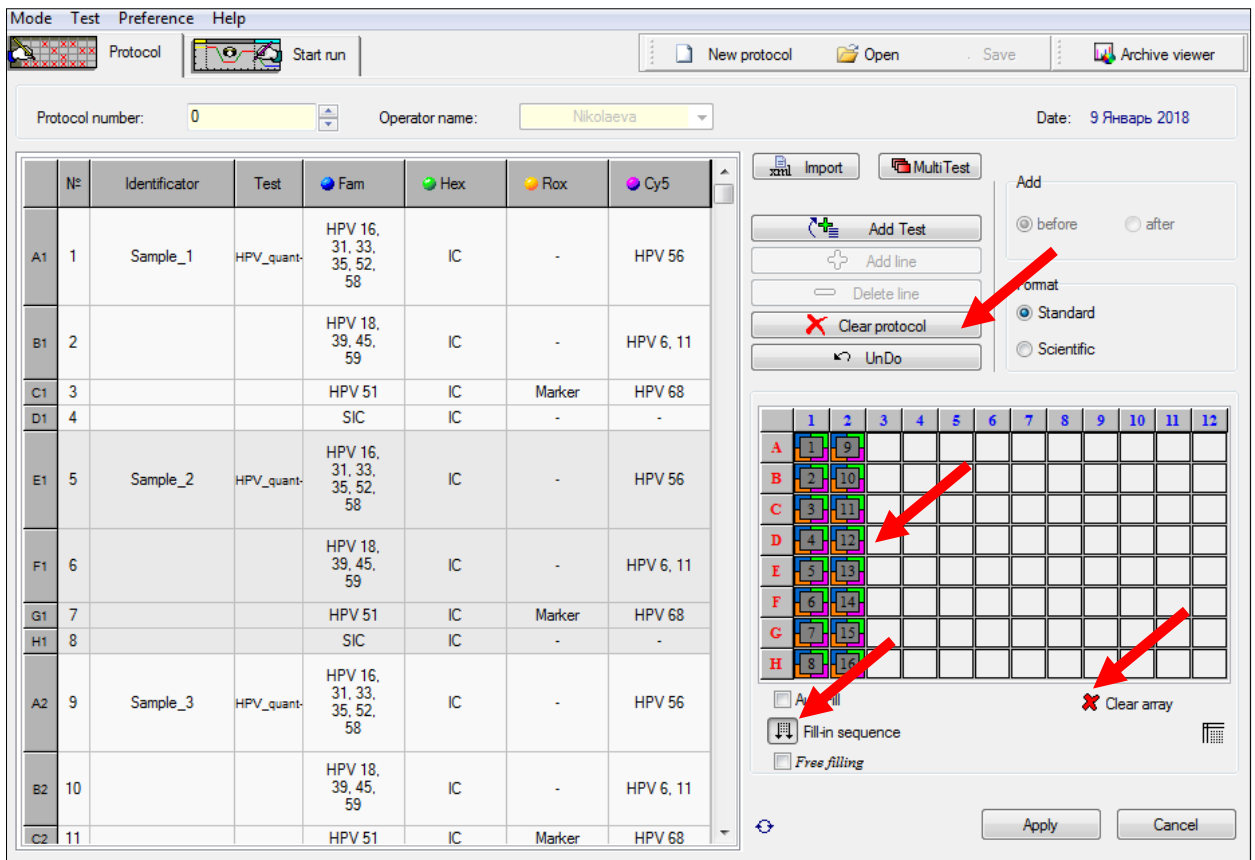
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

AutoFill Clear array

Fill in sequence Free filling

Apply Cancel

7.4.6. Define the position of strips in software interface according to position they were set in thermoblock (use ,  or  buttons).

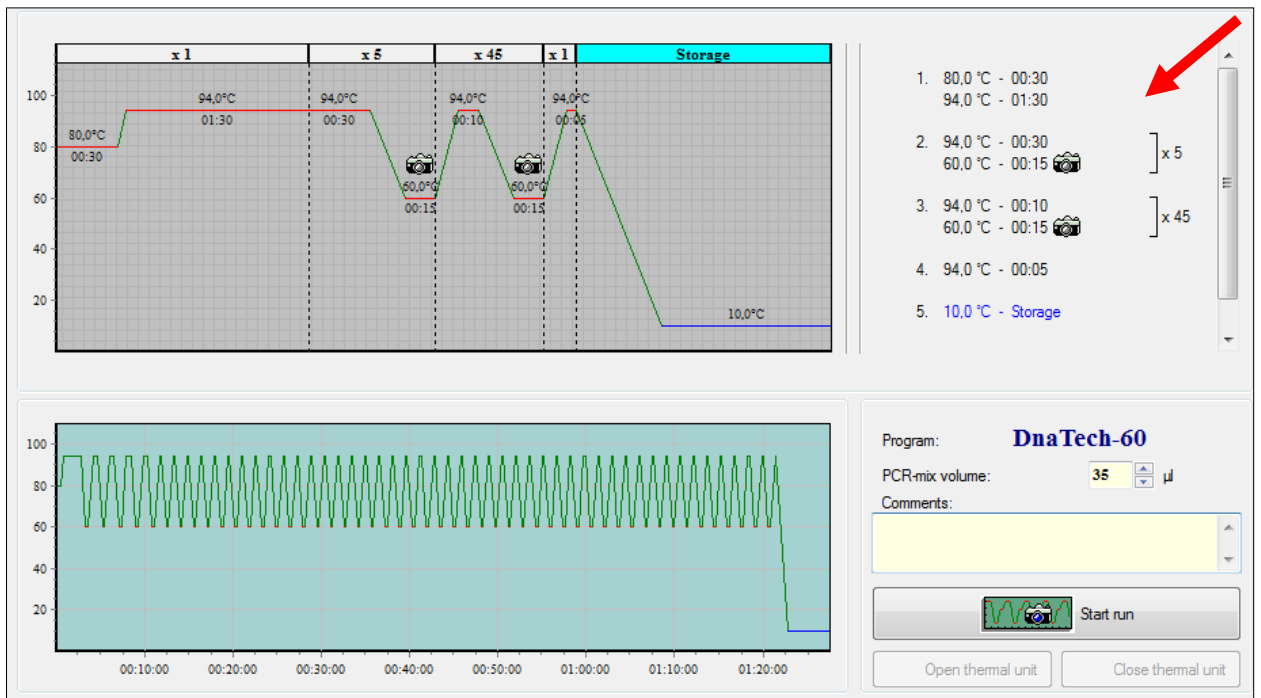


If the thermoblock is not completely filled, it is recommended to place the test tubes in the center of the thermoblock for evenly pressing of the tubes by the thermocycler hot lid.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C			1	2	3	4	5	6	7	8		
D			9	10	11	12	13	14	15	16		
E			17	18	19	20	21	22	23	24		
F			25	26	27	28	29	30	31	32		
G												
H												

7.4.7. Click "Apply" button in the lower-right corner of the "Protocol" window.

7.4.8. The cycling program will be displayed in the "Start run" window.



7.4.9. Click "Start run" button in the lower-right corner of the window

7.4.10. Write the name of the file and specify directory to save the data. The data will be saved to the operator's working directory by default (see p.7.3.1.)

8. DATA COLLECTION

The optical unit of the Real-time PCR machine collects data automatically as the cycling program proceeds.


The Real-time PCR machine detects and interprets results automatically.

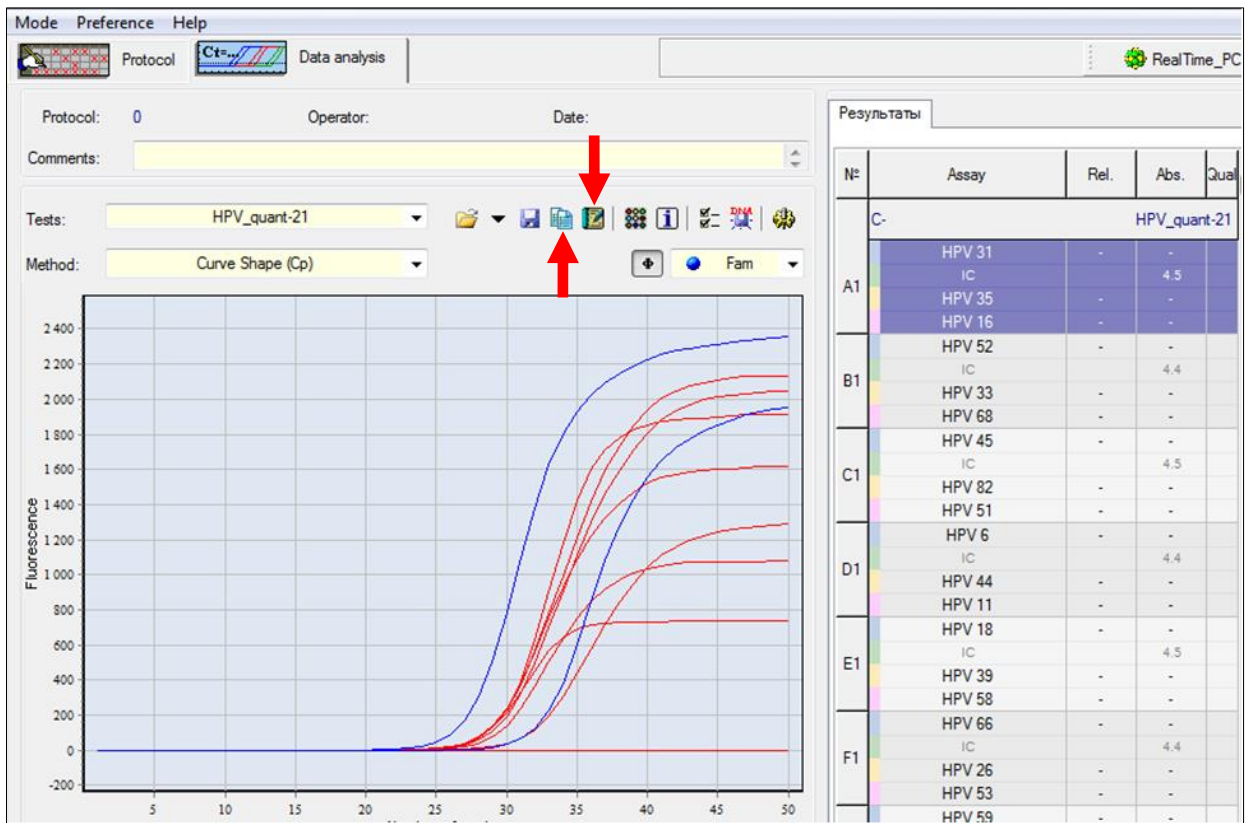
Upon completion of the run, a status screen will be displayed and the operator will be prompted to proceed to analysis. (User manual, Chapter 1 "Operating Procedure" p. 4.6).

Analysis will be performed by Real-Time PCR application automatically.

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.

To make a lab report click "Report"  button.

To make a specific report (recommended) on an instrument panel, press "Form of the answer" button  .



HPV

HPV quant-21

Date 21 Октябрь 2015, 12:20:21
 Number of tube
 Patient name
 Sex
 Age
 Organization
 Clinician name
 Comments



Information about laboratory

Sample ID: C+

№	Name of research	Results		
		Relative, Lq(X/SIC)*	Quantitative, Lq(copies/sample)	Qualitative
1	HPV 31	1.4	5.6	
2	HPV 35	1.7	5.9	
3	HPV 16	1.2	5.4	
4	HPV 52	0.8	5.0	
5	HPV 33	0.4	4.6	
6	HPV 68	0.8	5.0	
7	HPV 45	1.2	5.4	
8	HPV 82	0.6	4.8	
9	HPV 51	1.0	5.2	
10	HPV 6	1.3	5.5	
11	HPV 44	0.6	4.8	
12	HPV 11	1.5	5.7	
13	HPV 18	1.0	5.2	
14	HPV 39	0.8	5.0	
15	HPV 58	0.7	4.9	
16	HPV 66	1.1	5.3	
17	HPV 28	1.1	5.3	
18	HPV 53	0.5	4.7	
19	HPV 59	1.0	5.2	
20	HPV 56	1.3	5.5	
21	HPV 73	0.5	4.7	
22	SIC		4.2	

* HPV DNA copies/1 cell

Study was carried out by

Date
Signature

9. DATA ANALYSIS

- 9.1. The software analyzes data automatically. Software is provided together with DTlite, DTprime and DT-96 Real-time PCR machine manufacturer.
- 9.2. Upon completion of the run, software compares actual position of the strip with the real position of the marker (by mean of ROX dye label position) relative to position predefined by the operator. If there is a mismatch, the software warns the operator about it. Operator can either to rearrange the data obtained from each tube in right positions manually or to repeat the analysis of the given sample with the correct tubes order.
- 9.3. SIC value must be considered when analyzing results: SIC values lower than 4.0 should be considered as due to an insufficient amount of sample (see par. 3.2.), and the sampling procedure must be repeated.

9.4. 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 fixes 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.

Additional settings

Parameters of analysis | Type of analysis | Additional adjustment

1. Adjusting the relative analysis:
Copies of HPV DNA or cells

2. Type of results:


3. Take into account level to clinical value:
Estimation of the level to clinical value on result of the analysis:
Level of clinical significance: Copies of DNA

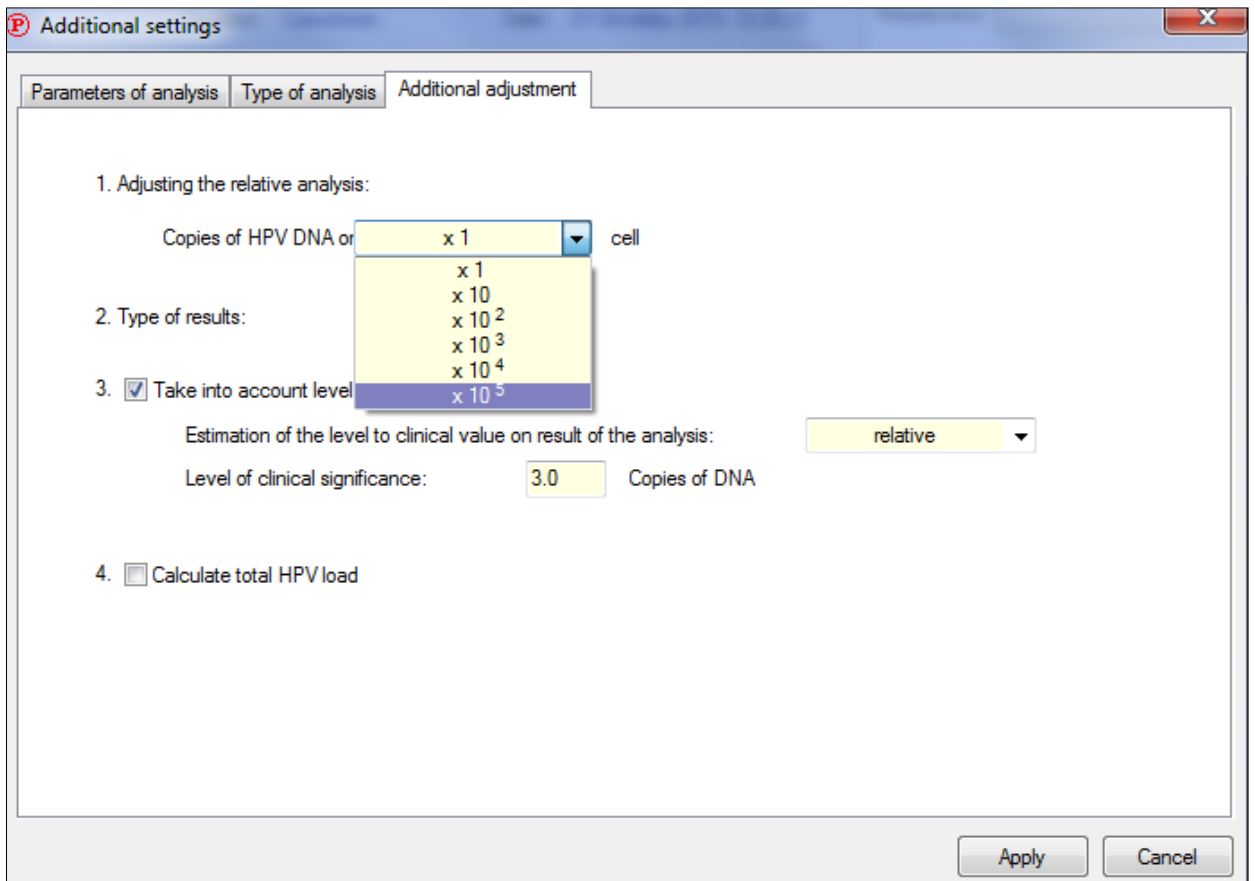
4. Calculate total HPV load

Apply Cancel

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 a single cell.



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 (see p. 7.3), only the presence or absence of HPV DNA in the sample is indicated.

- 9.5. In the absence of specific HPV signal and IC signal the program fixes 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).
- 9.6. For positive control samples, the program fixes a positive result. When negative results are obtained, all results of corresponding experiment should be considered as false. All samples must be reanalyzed.
- 9.7. For negative control samples, the program fixes 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.

10. TRANSPORTATION, STORAGE AND HANDLING REQUIREMENTS

10.1. Transportation

10.1.1. Transportation can be held by all types of roofed transport at the temperatures corresponding to the storage conditions of the components included in the kit.

10.1.2. Components of **HPV QUANT Quantitative Real-Time PCR Kit** transported with violation of the temperature regime must not be used.

10.2. Storage

- 10.2.1. All components of **HPV QUANT Quantitative Real-Time PCR Kit** must be stored at a temperature of 2 °C to 8 °C over the storage period.
- 10.2.2. Paraffin-sealed PCR-mixes must be stored in a dark place over the storage period.
- 10.2.3. Components of **HPV QUANT Quantitative Real-Time PCR Kit** stored with violation of the temperature regime must not be used.

10.3. Handling requirements

- 10.3.1. The **HPV QUANT Quantitative Real-Time PCR Kit** should be used in accordance with the current version of the approved instruction. We strongly recommend following the instructions to get robust and reliable results.
- 10.3.2. After unpacking, the kit components should be stored under the following conditions:
- the kit components should be stored at a temperature of 2 °C to 8 °C over the storage period;
 - paraffin-sealed PCR mixes should be stored in a dark place over the storage period.
- 10.3.3. An expired **HPV QUANT Quantitative Real-Time PCR Kit** must not be used.


















11. UTILIZATION GUIDELINE

- 11.1. Kits with expired shelf life, unused reagents and biomaterial should be disposed in accordance with the requirements of local legislation.

12. MANUFACTURER'S GUARANTEE

- 12.1. The conformity of **HPV QUANT Quantitative Real-Time PCR Kit** to the prescribed technical requirements is subject to compliance of storage, transport and operation conditions recommended by manufacturer.
- 12.2. Expiry date – 6 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions

13. KEY TO SYMBOLS

	Authorized representative in EU		In vitro diagnostic medical device		CE conformity marking
	Temperature limitation		Consult instructions for use		Catalogue number
	Upper limit of temperature		Manufacturer		Batch code
	Expiration date		Number of tests		Do not expose to sunlight
	Date of manufacture		Negative control		Positive control
	Caution		Version		

14. QUALITY POLICY

“DNA-Technology, Research&Production” LLC established the following goals to realize its quality policy focused on “customer satisfaction management” by improving the quality management system to comply with ISO9001:2000 and ISO13485:2003:

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

15. ADDRESS FOR ENQUIRY

With question regarding the quality of **HPV QUANT Quantitative Real-Time PCR Kit**, please, apply to official representative of manufacturer by address:

Address: 117587, Moscow, Varshavskoye sh., 125g building 6, DNA Technology

Contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93,

Phone/Fax: +7(495)640.17.71.

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

Address of manufacturer:

«DNA-Technology Research & Production», LLC, Russia, 142281, Moscow region, Protvino, Zheleznodorozhnaya street, 20.

Place of production:

Code of manufacturer is shown on the label (see the last number of the lot number):

1. «DNA-Technology Research & Production», LLC, Russia, 142281, Moscow region, Protvino, Zheleznodorozhnaya street, 20.
2. «DNA-Technology TS», LLC, Russia, 117246, Moscow, Nauchnyi proezd, 20, bld.4.

Authorized representative in EU:

OBELIS S.A

Registered Address:

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1030 Brussels,


Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

<http://www.obelis.net>

 539. Date of the statement: 08.08.2018

