

**Instructions for Use**

# **RealLine HPV HCR Screen Fla-Format**

**A QUALITATIVE ASSAY KIT FOR THE DETECTION OF DNA OF THE HIGH CARCINOGENIC RISK TYPES 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 AND 68 OF THE HUMAN PAPILLOMA VIRUS BY REAL TIME PCR**










*In-vitro* Diagnostics



|   |                      |                  |
|---|----------------------|------------------|
| <b>RealLine HPV HCR Screen (Fla-Format)</b> | <b>VBD8445</b>       | <b>100 Tests</b> |
| <b>valid from</b>                           | <b>February 2020</b> |                  |

## RealLine HPV HCR Screen Fla-Format

### Explanation of symbols used in labeling

|   |   |
|---|---|
|  | <i>In vitro</i> diagnostic medical device |
|  | Batch code                                |
|  | Catalogue number                          |
|  | Contains sufficient for <n> tests         |
|  | Use-by-date                               |
|  | Temperature limit                         |
|  | Consult instructions for use              |
|  | Keep away from sunlight                   |
|  | Manufacturer                              |



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#### Trademarks:

Rotor-Gene® is a registered trademark of Qiagen Group, Germany.

## RealLine HPV HCR Screen Fla-Format

### Table of content:

|  |    |
|--|----|
| 1. INTENDED USE  | 4  |
| 2. KIT CONTENTS  | 5  |
| 3. PRINCIPLE OF THE METHOD   | 6  |
| 4. SPECIFICATIONS  | 7  |
| 5. PRODUCT USE LIMITATIONS   | 7  |
| 6. WARNING AND PRECAUTIONS   | 8  |
| 7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED                      | 8  |
| 8. PREPARATION OF THE SPECIMEN   | 9  |
| 9. PROCEDURE   | 10 |
| 10. DATA ANALYSIS AND INTERPRETATION   | 12 |
| 11. STORAGE AND TRANSPORTATION   | 13 |
| ANNEX I: Settings for RealLine Cyclor and DT96:                                    | 14 |
| ANNEX II: Programming the device and analysis of results using Rotor-Gene cyclers: | 14 |

## RealLine HPV HCR Screen Fla-Format

**A QUALITATIVE ASSAY KIT FOR THE DETECTION OF DNA OF THE HIGH CARCINOGENIC RISK TYPES 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 AND 68 OF THE HUMAN PAPILLOMA VIRUS BY REAL TIME PCR**

*In vitro* Diagnostics

### 1. INTENDED USE

#### **Clinical information:**

Human Papilloma Viruses HPV are DNA-Viruses and in between are more than 100 different types are known. While the majority of HPVs cause no symptoms, some can cause low severe symptoms like warts and a few are known to cause cancer. HPV types that are more likely to lead to the development of cancer are referred as high-risk types HPV. High-risk HPV-types are known to cause the vast majority of cervical cancers which cause death in women with an annual incidence of around half a million and a mortality of almost 50 %.

The **RealLine HPV HCR Screen** assay kit is designed for detection of DNA of human papillomavirus (HPV) types:

- HPV type 16
- HPV type 18
- HPV type 31
- HPV type 33
- HPV type 35
- HPV type 39
- HPV type 45
- HPV type 51
- HPV type 52
- HPV type 56
- HPV type 58
- HPV type 59
- HPV type 66
- HPV type 68

The detection is subsequent to the isolation from clinical specimens using the extraction kits:

**RealLine DNA-Express (REF VBC8899)**

**RealLine DNA-Extraction 3 (REF VBC8889)**

**RealLine Extraction 100 (REF VBC8896)**

**RealLine HPV HCR Screen** kit is designed for the analysis of clinical materials: scrapings of the epithelial cells, semen, prostatic secretions, and urine. The assay is based on the real-time polymerase chain reaction (PCR) method with fluorescent detection of the amplified product.

**RealLine HPV HCR Screen** kit can be used in clinical practice.

The **Fla-format** Kit contains 2 x 10 vials with lyophilized mastermix, each vial with 10 reactions, for a volume of 50 µl per reaction. The kit contains reagents required for 100 tests, including the positive control samples.

The kit is designed for use with block cyclers iQ™ iCycler, iQ5™ iCycler, CFX96™ (Bio-Rad, USA), DT96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH); and rotor type cyclers Rotor-Gene® 3000, Rotor-Gene® 6000 and Rotor-Gene® Q (Qiagen, Germany).

## RealLine HPV HCR Screen Fla-Format

The use of:

- ! Extraction Kits for nucleic acids from clinical specimen from other supplier
- ! other real-time PCR devices
- ! appropriate reaction volumes, other than 50 µl

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

### 2. KIT CONTENTS

|  |                         |
|--|-------------------------|
| <b>Positive Control Sample (PC)</b>        | 2 vial, 1 ml            |
| <b>Master Mix No.1 (MM1)</b> , lyophilized | 10 tubes, 10 tests each |
| <b>Master Mix No.2 (MM2)</b> , lyophilized | 10 tubes, 10 tests each |
| <b>Recovery Solution (RS)</b>              | 4 vials, 2 ml each      |

## RealLine HPV HCR Screen Fla-Format

### 3. PRINCIPLE OF THE METHOD

Real time PCR is based on the detection of the fluorescence, produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is dual-labeled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescent signal increases due to the fluorescent dye and quencher separating by Taq DNA-polymerase exonuclease activity during amplification. PCR process consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value - Ct – is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal rises significantly above the background fluorescence. Increased fluorescence signal is due to the use of a specific for given CMV DNA sequence DNA hybridization probe that in the course of reaction binds with one of the DNA strands, also providing additional specificity of the method. DNA probe comprises of a fluorescent dye at the 5' end and of fluorescence quencher at the 3' end which significantly reduces the fluorescence intensity. During the polymerase synthesis of the complementary strand, due to the 5'-3' nuclease activity of Taq DNA polymerase the probe is cleaved from the 5'-terminus and separation of the quencher and the dye occurs, resulting in the increase the fluorescence signal due to accumulation of the reaction product. Fluorescence intensity detected depends on initial quantity of pathogen DNA template in the sample.

The use of Internal Control (IC) prevents generation of false negative results associated with possible loss of DNA template during specimen preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC template should be added in each single sample (including control samples) prior to DNA extraction procedure. The amplification and detection of IC does not influence the sensitivity or specificity of the target DNA PCR.

Note: IC is a component of the NA extraction kits of RealLine series. Internal Control is added to the sample during NA isolation step and is used throughout the whole process of NA extraction, amplification, detection.

Two tubes (RMM1 and MM2) are used in the analysis of the content of HPV HCR DNA for each sample.

## RealLine HPV HCR Screen Fla-Format

### 4. SPECIFICATIONS

#### 4.1. Sensitivity:

Sensitivity of detection of DNA HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (on four samples, containing 100 DNA copies, prepared from Standard Reference Samples) equals 100 %.

#### 4.2. Specificity:

Specificity of detection of DNA HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (using Standard Reference Panel of negative DNA-extracts) equals 100 %.

#### 4.3. Diagnostic evaluation:

##### Diagnostic sensitivity

Diagnostic sensitivity of DNA detection of human papilloma virus of high cancer risk: clinical trials, conducted on 95 positive samples showed a 100 % sensitivity (range 97 % -100 %, with 90 % confidence level).

##### Diagnostic specificity

Diagnostic specificity of DNA detection of human papilloma virus of high cancer risk: clinical trials, conducted on 54 negative samples showed a 100 % specificity (range 95 % -100 %, with 90 % confidence level).

### 5. PRODUCT USE LIMITATIONS

- This assay must not be used on the clinical specimen directly. Appropriate nucleic acids extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be comparable.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with Human Papilloma Virus infections. The kit may be used for screening purposes.
- Diagnostic sensitivity of the kit may vary depending on the pathogen prevalence and characteristics of the enrolled cohort.
- Reliable results depend on adequate specimen sampling.
- Positive results indicate active or asymptomatic infection; clinical history and symptoms should be taken into account.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the Human Papilloma Virus genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The kit is not intended to replace culture and other methods (e.g., cervical exam) for diagnosis of infections.

## RealLine HPV HCR Screen Fla-Format

### 6. WARNING AND PRECAUTIONS

- ☞ For in vitro use only.
- ☞ The kits must be used by skilled personnel only.
- ☞ When handling the kit, follow the national safety requirements for working with pathogens.
- ☞ To prevent contamination, the stages of DNA isolation and PCR test run must be spatially separated.
- ☞ Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent vials.
- ☞ Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- ☞ Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- ☞ The use of sterile disposable pipette tips is recommended.
- ☞ Never use the same tips for different samples.
- ☞ Do not pool reagents from different lots or from different vials of the same lot.
- ☞ Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- ☞ Do not use the kit after the expiration date at the side label of the kit.

### 7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kit: **RealLine DNA-Express, RealLine DNA-Extraction 2, RealLine DNA-Extraction 3 or RealLine Extraction 100,**
- Internal Control reagent (VBC8881) and Negative Control Sample, if the kit is used with the extraction kits of other supplier;
- Plates or Tubes suitable for the used device with caps or a sealing foil for PCR
- laminar safety box;
- refrigerator;
- half-automatic variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves;
- disposable pipette tips with aerosol barrier;
- biohazard waste container;
- razor or scalpel



## RealLine HPV HCR Screen Fla-Format

### 8. PREPARATION OF THE SPECIMEN

The assay is performed on extracted DNA samples obtained from the clinical material using one of the DNA extraction kits listed in p.1, according to the Instruction Manual to the kit.

*Each group of samples undergoing the procedure of NA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** that is a component of the RealLine NA extraction kits.*

*The **RealLine Internal Control** (VBC8881 or component of the RealLine NA Extraction Kits) Sample must be added to the extractions procedure to all samples and controls.*

When using a kit of another supplier for the extraction of nucleic acids as recommended in p. 1., add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of the Negative Control Sample
- For the PC use **70 µl** of Negative Control Sample and **30 µl** of Positive Control to the tube marked PC.

Prepare the samples for the assay using **RealLine DNA-Express**, **RealLine DNA-Extraction 2**, **RealLine DNA-Extraction 3** or **RealLine Extraction 100** extraction kits according their instruction manuals.

If samples of isolated DNA were stored frozen prior the assay, thaw them and keep at least 30 minutes at a temperature of (18 – 25) °C.

*The isolated DNA can be stored at (2 – 8) °C for 24 hours*

*After initial opening shelf life of Positive Control sample at (2 – 8) °C is 1 month or for 50 µl aliquots 3 month at (-18 ... -60) °C*

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## RealLine HPV HCR Screen Fla-Format

### 9. PROCEDURE

#### 9.1. Preparation of the kit components

Prior to the test, take the kit out of the refrigerator, open the package, take the necessary number of tubes with the **Master Mix for PCR (MM)**, taking into account prepared specimens and control samples. 1 NC and 1 PC for each test is required. Keep the tubes at (18 – 25) °C for at least 30 min.

Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

*Attention! Each tube is intended for 10 tests.*

*After initial opening, store MM at (2 – 8) °C for no more than 3 months.*

To prepare diluted Master Mix, add **300 µl of Recovery Solution RS** to each tube with MM. Mix gently, keep at (18 - 25) °C during 15 min, and mix thoroughly one more time. Collect the tube contents by brief centrifugation.

*Store diluted MM at (2 – 8) °C for no more than 7 days.*

*After initial opening, store RS at (2 – 8) °C for no more than 3 months.*

**9.2.** Prepare an appropriate number of 0.2 ml tubes. Label each tube for each specimen and control (each specimen requires two tubes with MM1 and MM2).

**Attention!** Labels should be placed on the caps of tubes for Rotor-Gene 3000/6000/Q devices. For iQ5 iCycler, CFX96, DT-96 PCR devices labels should be placed on the lateral side of the tubes.

**9.3.** Add **25 µl** of prepared Master Mix to each 0.2 ml tube.

**9.4.** Add **25 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter (each sample must be added to 2 tubes, containing MM1 and MM2). Tightly close the tubes.

**9.5.** Place the tubes into the real-time PCR system.

**9.6.** Program the device for HPV DNA amplification of specific fragments of DNA, and detection of fluorescence signals. The thermocycler programming carried out in accordance with the instruction to the device.

**9.7.** Program the amplification reaction protocol:

**For Rotor-Gene® 3000 (6000, Q):**

|         |       |        |           |
|---------|-------|--------|-----------|
| Step 1: | 50°C  | 2 min  | 50 cycles |
| Step 2: | 95°C  | 2 min  |           |
| Step 3: | 94°C  | 10 sec |           |
|         | 60°C* | 25 sec |           |

\* Measure the fluorescence at 60°C.

## RealLine HPV HCR Screen Fla-Format

### For RealLine Cyclers, iQ™ iCycler, iQ™5 iCycler, CFX96™, DT-96:

|         |       |        |           |
|---------|-------|--------|-----------|
| Step 1: | 50°C  | 2 min  | 50 cycles |
| Step 2: | 95°C  | 2 min  |           |
| Step 3: | 94°C  | 10 sec |           |
|         | 60°C* | 20 sec |           |

\* Measure the fluorescence at 60°C

### 9.8. Select the channels for the detection of DNA amplification results:

#### MM1:

For registration of IC DNA amplification signal a DNA hybridization probe labeled with following fluorophore is applied

- **FAM:** for iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000
- **Green:** for Rotor-Gene 6000, Rotor-Gene Q

For registration of high carcinogenic risk HPV DNA amplification signal, DNA hybridization probes labeled with fluorophores **HEX/JOE/Yellow** and **ROX/Orange** are applied.

Selected detection channels:

- **HEX, ROX:** for iQ5 iCycler, CFX96 and DT-96
- **JOE, ROX:** for Rotor-Gene 3000
- **Yellow, Orange:** for Rotor-Gene 6000, Rotor-Gene Q

#### MM2:

For registration of IC DNA amplification signal a DNA hybridization probe labeled with following fluorophore is applied

- **FAM:** for iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000
- **Green** for Rotor-Gene 6000, Rotor-Gene Q

For registration of high carcinogenic risk HPV DNA amplification signal, DNA hybridization probe labeled with fluorophore **ROX/Orange** is applied:

- **ROX:** for iQ5 iCycler, CFX96 and DT-96
- **ROX:** for Rotor-Gene 3000
- **Orange:** for Rotor-Gene 6000, Rotor-Gene Q

### 9.9. Program the position of tubes with specimens, positive and negative controls according to the instruction manuals to the used device.

### 9.10. Run the program and conduct the amplification reaction with the registration of fluorescence in real-time.

## RealLine HPV HCR Screen Fla-Format

### 10. DATA ANALYSIS AND INTERPRETATION

10.1 For **PC** in **MM1** the program should detect:

- increase of the IC DNA amplification signal (channel **FAM/Green**) and determine the threshold cycle, IC Ct;
- increase of the HPV DNA amplification signal (channels **HEX/ JOE/ Yellow, ROX/ Orange**) and determine the PC **Ct** value for each channel.

For **PC** in **MM2** the program should detect:

- increase of the IC DNA amplification signal (channel **FAM/Green**) and determine the threshold cycle, IC Ct;
- increase of the HPV DNA amplification signal (channel **ROX/ Orange**) and determine the PC **Ct** value for each channel.

10.2 For **NC** in each of **MM1** and **MM2** the program should detect the increase of the amplification signal of IC DNA (channel **FAM/Green**) and determine the threshold cycle, IC **Ct**. No **HEX/JOE/ Yellow, ROX/ Orange** fluorescent increase should appear in **MM1** and no **ROX/ Orange** fluorescent increase in **MM2** (*no HPV DNA amplification*).

10.3 For each sample in **MM1** and **MM2** an increase of the amplification signal of **IC DNA** (channel **FAM/Green**) should be detected and the IC **Ct** determined. .

10.4 Calculate  $(IC\ Ct)_{av}$  as an average IC **Ct** of all analysed samples (including PC and NC) independently for each of **MM1** and **MM2**. IC Ct values that differ by more than 2 from the  $(IC\ Ct)_{av}$  should be ignored. Recalculate the  $(IC\ Ct)_{av}$  for the remaining values after the screening.

10.5 The sample is considered **negative** (not containing HPV HCR DNA), if **Ct** value via **HEX/JOE/ Yellow, ROX/ Orange** channels in **MM1** and via **ROX/ Orange** channel in **MM2** for this sample is **above 40** or is not determined.

If IC **Ct** value for such sample differs from the  $(IC\ Ct)_{av}$  value by more than 2, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.

10.6 **For MM1:** The sample is considered **positive**, i.e. contains *HPV HCR DNA*, when **Ct** value via **HEX/JOE/ Yellow** and / or **ROX/ Orange** channels is **less than or equals to 40**.

**For MM2:** The sample is considered **positive**, i.e. contains *HPV HCR DNA*, when **Ct** value via **ROX/ Orange** channels is **less than or equals to 40**.

## RealLine HPV HCR Screen Fla-Format

- 10.7** If Ct value for NC through **HEX/JOE/Yellow, ROX/Orange** channels for **MM1** or **ROX/Orange** for **MM2** is less than or equal to **40**, this indicates the presence of contamination. In this case all positive results of this individual PCR run are considered equivocal. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all samples of this run that were identified as positive. Samples that showed negative results in this run should be considered as negative.

**The sample is positive when positive signals are detected in in one or in both Mastermixes!**

The occurrence of two or more different positive Genotypes is often found in patient samples!

For genotyping of the HPV types use the RealLine HPV HCR Genotype kits (REF VBD8478, VBD8479, VBD8482) or ask us for more information: [techsupport@bioron.de](mailto:techsupport@bioron.de)

### 11. STORAGE AND TRANSPORTATION

- Store and transport the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport at (2 - 8) °C .Transportation at 25 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction Manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.
- **Storage and shelf life of solutions and components of the kit after initial opening:**
  - Positive Control sample: 1 month at (2 - 8) °C or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months.
  - Ready Master Mix (MM): unused MM at (2 - 8) °C for no more than 3 months
  - Diluted MM: at (2 - 8) °C for 7 days in a dark surrounding
  - Recovery Solution: at (2 - 8) °C for 3 months.

Technical support: [techsupport@bioron.de](mailto:techsupport@bioron.de)

## RealLine HPV HCR Screen Fla-Format

### ANNEX I: Settings for RealLine Cyclers and DT96:

for these cyclers the measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition:**

- **FAM** to **250**
- **HEX** and **ROX** to **1000**

Confirm that the current exposure value is saved by pressing **YES**

**Attention!** The specified exposure values are applicable only for RealLine kits and, if necessary, must be changed for other purposes.

### ANNEX II: Programming the device and analysis of results using Rotor-Gene cyclers:

#### Rotor-Gene 3000, Rotor-Gene 6000 (Corbett research, Australia), Rotor-Gene Q (Qiagen, Germany)

Hereinafter, detection channels and terms corresponding to different versions of devices and software are listed in the following order: Rotor-Gene 3000 (Rotor-Gene 6000, Rotor-Gene Q).

- 1) Click **New** button.
- 2) Select an **Advanced** template from the tab of the New Run wizard. Click **New** button.
- 3) Select **36-Well Rotor** type, check that No Domed 0.2 ml Tubes are used. Click **Next** button.
- 4) In the new window, determine Reaction volume as **50 µl**. Click **Next** button.
- 5) The temperature profile of real time PCR should be set. Click **Edit Profile** button.

|         |       |        |           |
|---------|-------|--------|-----------|
| Step 1: | 50°C  | 2min   | 50 cycles |
| Step 2: | 95°C  | 2min   |           |
| Step 3: | 94°C  | 10 sec |           |
|         | 60°C* | 25 sec |           |

\* Measure the fluorescence at 60°C

- 6) Then temperature profile is set, click **OK** button.
- 7) In the **New Run Wizard** window click **Calibrate (Gain optimization)** button. The window **Auto Gain Calibration Setup** opens. In the line **Channel Settings** choose **ROX (Orange)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**. In the line **Channel Settings** choose **JOE (Yellow)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**. In the line **Channel Settings** choose **FAM (Green)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**.
- 8) Tick off **Perform Calibration Before 1st Acquisition**. Click **Close** button.
- 9) Click **Next** button, start the amplification process by clicking **Start Run** button.
- 10) Save a file in the Rotor-Gene/templates folder, named RealLine with \*.ret extension. In subsequent work RealLine template would be presented in New run wizard.
- 11) Save reaction result file with Rotor-Gene Run File \*.rex extension.
- 12) Record the positions of the controls and specimens according to the instruction manual of the operating device. Click **Start run** button.

## RealLine HPV HCR Screen Fla-Format

### Results of IC DNA amplification

- 1) Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. FAM** («Cycling A. Green»), click **Show** button.
- 2) Click **OK** button, and cancel automatic **Threshold** determination.
- 3) Click **Linear scale** button. Settings should change to **Log. scale**.
- 4) In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- 5) Click **More Settings (Outlier Removal) button**, determine **NTC threshold** value as **5 %**.
- 6) In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
- 7) In the result table (**Quant. Results window**) **Ct** will be displayed.

### Results of HPV DNA amplification

- 1) Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. JOE** (*Cycling A. Yellow*) click, **Show** button.
- 2) Click **OK** button, and cancel automatic **Threshold** determination.
- 3) Click **Linear scale** button. Settings should change to **Log. scale**.
- 4) In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- 5) Click **More Settings (Outlier Removal) button**, determine **NTC threshold** value as **5 %**.
- 6) In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
- 7) In the result table (**Quant. Results window**) **Ct** will be displayed.

### Results of HPV DNA amplification

- 1) Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. ROX** (*Cycling A. Orange*) click, **Show** button.
- 2) Click **OK** button, and cancel automatic **Threshold** determination.
- 3) Click **Linear scale** button. Settings should change to **Log. scale**.
- 4) In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- 5) Click **More Settings (Outlier Removal) button**, determine **NTC threshold** value as **5 %**.
- 6) In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
- 7) In the result table (**Quant. Results window**) **Ct** will be displayed.

**RealLine HPV HCR Screen  
Fla-Format**

