

Instructions for use

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

AN ASSAY KIT FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* DNA BY REAL-TIME PCR

In vitro Diagnostics



RealLine Chlamydia trachomatis / Neisseria gonorrhoeae (Fla-Format)	VBD0458	100 Tests
valid from	September 2019	

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae 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 Chlamydia trachomatis /
Neisseria gonorrhoeae
Fla-Format**

Table of content:

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

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

AN ASSAY KIT FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* DNA BY REAL-TIME PCR

In vitro Diagnostics

1. INTRODUCTION AND INTENDED USE

1.1. Pathogen information

Chlamydia trachomatis is a Gram-negative, ovoid, non-motile bacterium from the genus *Chlamydia*. It is an obligate intracellular parasite, for which humans are the only host [1]. Chlamydia is one of the most common sexually transmitted diseases worldwide. Infections are most common among those between the ages of 15 and 25 and are more common in women than men. The young women have the highest prevalence of genitourinary *C. trachomatis* infections. Most infected women (more than 70%) and about 50% of infected men are asymptomatic [2]. Women infected with chlamydia are up to three times more likely to become infected with HIV, if exposed [3]. In men, the most common manifestation of *C. trachomatis* infection is inflammation of the urethra (about 50% of cases) [4]. The untreated infection may result in epididymitis, which in some cases can lead to fertility problems. Neonates born of infected mothers can develop inclusion conjunctivitis, nasopharyngeal infections and pneumonia due to *C. trachomatis* [5]. Definitive diagnosis is very important for infected person and his/her partner(s). As many people in the population are infected and the significant proportion of infection is asymptomatic, the diagnosis is often established by screening, which is recommended to perform regularly in sexually active young women, in other people from higher risk groups, and at the first prenatal visit [6]. Most *C. trachomatis* strains have a multicopy extrachromosomal cryptic plasmid, which is widely used as the target for PCR testing. As some *C. trachomatis* isolates are reported not to carry cryptic plasmid or have deletion(s) in it [7], “RealLine Chlamydia trachomatis / Neisseria gonorrhoeae” assay kit detects two DNA fragments from *gyrA* gene and cryptic plasmid, specific to *C. trachomatis* species.

Neisseria gonorrhoeae is a species of Gram-negative diplococci non-motile bacteria from the genus *Neisseria*. It causes a sexually transmitted disease, gonorrhoea, which remains a major global public health concern since many identified strains of *N. gonorrhoeae* are resistant to most available antibiotics [8]. Infection generally localizes in the genital mucosa, but can be found also in ocular, nasopharyngeal and anal mucosa [9-11]. Symptoms include purulent exudates from penis and painful urination in men, and abnormal vaginal discharge in women, although often the infection is asymptomatic. Complications from untreated genital-tract-localized infection can cause pelvic inflammatory disease, ectopic pregnancy and infertility in women, and epididymitis and infertility in men [12, 13]. Rarely, disseminated gonococcal infection develops in untreated patients resulting in infectious arthritis and endocarditis [14]. Perinatal transmission may occur during childbirth leading to neonatal conjunctivitis and blindness [15]. In the past, gonorrhoea was diagnosed by a Gram stain of the patient’s purulent exudate; nowadays, nucleic-acid-based assays are used, but often the diagnosis is confirmed by culture methods [8]. “RealLine Chlamydia trachomatis / Neisseria gonorrhoeae” assay kit detects a part of PivNG (pilin gene inverting protein homolog) gene sequence, specific to *N. gonorrhoeae*.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

1.2. Intended use

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae assay kit is intended for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in clinical specimens: urine, semen, prostate fluid, swabs of the epithelial cells (urethral, cervical, vaginal), using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

The extraction of DNA from clinical materials can be performed using the extraction kits:

- **RealLine DNA-Express (REF VBC8899)**
- **RealLine DNA-Extraction 2 (REF VBC8897)**
- **RealLine DNA-Extraction 3 (REF VBC8889)**
- **RealLine Extraction 100 (REF VBC8896)**

When using DNA extraction kits of other manufacturers it is highly recommended to use Internal Control sample (IC) (VBC8881, BIORON Diagnostics)

The results of PCR analysis are taken into account in complex diagnostics of disease.

The Fla-format Kit of the kit is intended for use with rotor-type PCR cyclers: Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia) and Rotor-Gene Q (Qiagen, Germany) and block-type PCR cyclers: iQ5 iCycler, CFX96 (Bio-Rad, USA) and different modifications of DT-48/96 / DTprime, DT-48 / DTlite (DNA-Technology, Russia), RealLine Cyclers (BIORON Diagnostics GmbH, Germany) and SaCycler-96 (Sacace Biotechnologies s.r.l., Italy).

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

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.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

2. KIT CONTENTS

Universal Positive Control Sample (PC) -	1 vial, 1 ml
Master Mix for PCR (MM), lyophilized	10 tubes, 10 tests each
Recovery Solution (RS)	2 vials, 2 ml each

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 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 DNA extraction kits of RealLine series. Internal Control is added to the specimen during DNA isolation step and is used throughout the whole process of DNA extraction, amplification, detection.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

4. SPECIFICATIONS

4.1. Analytical Specificity:

Analytical specificity of “RealLine Chlamydia trachomatis / Neisseria gonorrhoeae” assay kit is ensured by the specific primers and probes. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Analytical specificity of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA detection is determined using the Standard Reference Panel of negative DNA extracts as percentage of samples determined by the kit as negative. Analytical specificity equals 100%.

4.2. Analytical sensitivity:

Analytical sensitivity of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA detection is determined on five samples containing 100 copies of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA per sample prepared from Standard Reference Samples (*Chlamydia trachomatis* DNA SRS and *Neisseria gonorrhoeae* DNA SRS) as percentage of samples determined by the kit as positive. Analytical sensitivity equals 100%.

4.3. Diagnostic evaluation:

Diagnostic sensitivity of *Chlamydia trachomatis* DNA detection was evaluated on a collection of 415 clinical samples: 200 urine samples (123 from men and 77 from women), 133 cervical swabs and 82 urethra swabs from patients with STI characteristic symptoms. Diagnostic sensitivity equaled 96.6%.

CE-marked reference kit confirmed the presence of *C. trachomatis* DNA in the samples.

Diagnostic sensitivity of *Neisseria gonorrhoeae* DNA detection was evaluated on a collection of 228 clinical samples: 78 urine samples (55 from men and 23 from women), 129 cervical swabs and 21 urethra swabs from patients with STI characteristic symptoms. Diagnostic sensitivity equaled 98.4%.

CE-marked reference kit confirmed the presence of *N. gonorrhoeae* DNA in the samples.

All discordant results obtained concern samples with low content of detectable DNA fragment that falls outside the reliable detection limit of the kits.

Diagnostic specificity of *C. trachomatis* and *N. gonorrhoeae* DNA detection was evaluated on a collection of 200 urine samples and mucosal swabs from healthy donors. 120 Urine samples (30 from men and 90 from women) and 80 mucosal swabs (11 from men and 69 from women) were analyzed. Obtained results were in full agreement with CE-marked reference kits.

Diagnostic specificity equaled 100%.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

5. 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 *C. trachomatis* and *N. gonorrhoeae* 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 *C. trachomatis* and *N. gonorrhoeae* 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 urogenital infection.
- All results positive for *Neisseria gonorrhoeae* DNA can be confirmed by “**RealLine Neisseria gonorrhoeae T2**” (VBD4494), or other kit that detects target different from PivNG gene sequence

NOTES:

Internal analyses show that 0.002% (2 out of 100,000) clinical samples positive for Neisseria gonorrhoeae may give false positive results. In nasopharyngeal samples, cross-reactions with nosocomial bacteria such as Neisseria subflava, N. flavescens may occur. The statistical frequency of false positive results in these samples is approximately 2.5 % of the positive samples [16]. Further information can be found on the Robert-Koch-Institute website at:

https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Gonorrhoe.html

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae 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 ribonuclease 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.
- ☞ To conduct amplification reaction with real-time PCR products detection, use only disposable tips with filters.
- ☞ 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 total expiration date at the side label of the box.

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.
- PCR Plates or tubes suitable for the used device with caps or a sealing foil for PCR
- Laminar safety box;
- Refrigerator;
- Microcentrifuge;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol filter;
- Biohazard waste container;
- Scalpel or razor
- Racks for 2 ml and 0.2 ml tubes

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae 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 chapter 1.2, according to the Instruction Manual to the kit. If an extraction kit with magnetic particles is used, keep the tubes with extracted DNA in the magnetic rack.

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

We strongly recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure.

When using kits of another supplier for the extraction of nucleic acids as recommended in chapter 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.

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.

Store the extracted DNA at (2–8) °C for no more than 24 hours.

After initial opening, store Positive Control sample at (2 – 8) °C for no more than 1 month or in 50 µl aliquots 3 month at (-18 ... -24) °C for no more than 3 month.

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. For each test 1 NC and 1 PC are 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 with MM is intended for 10 tests.

After initial opening of the package, 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 for 15 min, and mix again.

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.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

9.2 Prepare an appropriate number of 0.2 ml tubes. Label each tube for each specimen and control sample.

Attention! Labels should be placed on the caps of tubes for rotor-type cyclers. For block-type cyclers 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. Do not touch the pellet! Tightly close the tubes.

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

9.6 Program real time PCR system as follows:

For Rotor-Gene® 3000 (6000, Q):

Step 1:	50°C	2 min	
Step 2:	95°C	2 min	
Step 3:	94°C	10 sec	50 cycles
	60°C*	40 sec	

* Measure the fluorescence at 60°C.

For, iQ™ iCycler, iQ™5 iCycler, CFX96™, DT-48/96, RealLine Cycler:

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

* Measure the fluorescence at 60°C

9.7 Select the amplification detection channels:

- Collect data through **FAM** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-48/96, Rotor-Gene 3000) or **Green** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **IC DNA**;
- Collect data through **ROX** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-48/96, Rotor-Gene 3000), and **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **Neisseria gonorrhoeae** DNA;
- Collect data through **HEX** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-48/96, Rotor-Gene 3000), and **JOE / Yellow** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **Chlamydia trachomatis** DNA;

9.8 Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use.

9.9 Run the program.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

10. DATA ANALYSIS AND INTERPRETATION

10.1 For **Positive Control** the program should detect:

- Increase of the **IC DNA** amplification signal (channel **FAM/Green**) and determine the threshold cycle, **IC Ct**;
- Increase of the **Chlamydia trachomatis** DNA amplification signal (channel **HEX/JOE/Yellow**) and determine the **PC Ct** value;
- Increase of the **Neisseria gonorrhoeae** DNA amplification signal (channel **ROX/Orange**) and determine the **PC Ct** value.

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM/Green**) and determine the threshold cycle **IC Ct**. No **ROX/Orange** or **HEX/JOE/Yellow** fluorescent increase should appear (*no Chlamydia trachomatis or Neisseria gonorrhoeae DNA amplification*).

10.3 For each sample the program should detect the increase of the amplification signal of IC DNA (channel **FAM/Green**) and determine **IC Ct**.

10.4 Calculate $(IC\ Ct)_{av}$ as an average **IC Ct** of all analyzed samples (including PC and NC). **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 specimen is considered **positive**, i.e. containing *Neisseria gonorrhoeae* DNA, if **Ct** value through **ROX/Orange** channel for this specimen is **less than or equals to 40**.

The specimen is considered **positive**, i.e. containing *Chlamydia trachomatis* DNA, if **Ct** value through **HEX/JOE/Yellow** channel for this specimen is **less than or equals to 40**.

10.6 The specimen is considered negative, if **Ct** value through **ROX/Orange** and **HEX/JOE/Yellow** channels for this specimen is **above 40 or is not determined**. If **IC Ct** value for such specimen differs from the $(IC\ Ct)_{av}$ value by more than 2, the result is regarded as **equivocal**. A repeated analysis of the specimen, starting from the DNA extraction step is required.

10.7 If **Ct** value for NC through **ROX/Orange** or **HEX/JOE/Yellow** channel is **less than or equal to 40**, it 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. Repeat the analysis of all specimens of this run that were identified as positive. Specimens that showed negative results in this run should be considered negative.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport the kit at (2 – 8) °C, transportation for up to 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.

12. REFERENCES

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RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

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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*	40 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**.

RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

- 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.

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 Chlamydia trachomatis 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 **10%** .
- 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 Neisseria gonorrhoeae 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.

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**RealLine Chlamydia trachomatis /
Neisseria gonorrhoeae
Fla-Format**

