RealLine Neisseria gonorrhoeae Fla-Format

Instructions for Use

RealLine Neisseria gonorrhoeae Fla-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *NEISSERIA GONORRHOEAE* DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Neisseria gonorrhoeae (Fla-Format)	**VBD4496**	**100 Tests**
valid from	**September 2019**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
	Content of number of tests
	Expiry date
	Temperature limitation
	Consult instructions for use
	Manufacturer
	Keep out of sunlight

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1. INTRODUCTION AND INTENDED USE

1.1. Clinical information:

Neisseria gonorrhoeae is a species of Gram-negative diplococci non-motile bacteria from the genus Neisseria. It causes a sexually transmitted disease, gonorrhea, which remains a major global public health concern since many identified strains of *N. gonorrhoeae* are resistant to most available antibiotics [1]. Infection generally localizes in the genital mucosa, but can be found also in ocular, nasopharyngeal and anal mucosa [2-4]. 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 [5, 6]. Rarely, disseminated gonococcal infection develops in untreated patients resulting in infectious arthritis and endocarditis [7]. Perinatal transmission may occur during childbirth leading to neonatal conjunctivitis and blindness [8]. In the past, gonorrhea 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 [1]. "RealLine Neisseria gonorrhoeae" assay kit detects a part of PivNG (pilin gene inverting protein homolog) gene sequence, specific to *N. gonorrhoeae*.

1.2. Intended use

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

The RealLine Neisseria gonorrhoeae (Fla-format) assay kit is designed to detect *Neisseria gonorrhoeae* DNA isolated from clinical specimens using one of the following extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

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

This Kit contains 10 vials with the 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 control samples.

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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-96 / DTprime, DT-48 / DTlite (DNA-Technology, Russia), RealLine Cyclers (BIORON Diagnostics GmbH, Germany) and SaCycler-96 (Sacace Biotechnologies s.r.l., Italy).

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**

have 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

Universal Positive Control sample (PC)	1 vial, 1 ml
Master Mix (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. Ct depends on initial quantity of pathogen DNA template.
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.

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4. SPECIFICATIONS

4.1. **Specificity** of *Neisseria gonorrhoeae* DNA detection is determined using the Standard Reference Panel of negative DNA-extracts, as a percentage of the samples determined by the kit as negative. Specificity equals 100 %.

4.2. **Sensitivity** is determined on five samples containing 100 copies of *Neisseria gonorrhoeae* DNA per a sample, prepared from the Standard Reference Sample, as a percentage of the samples determined by the kit as positive. Sensitivity equals 100 %.

4.3. **Diagnostic sensitivity** of the *Neisseria gonorrhoeae* DNA detection: clinical tests performed on 94 positive samples showed 100 % sensitivity (interval 97 % -100 % with a confidence level of 90 %);

4.4. **Diagnostic specificity** of the *Neisseria gonorrhoeae* DNA detection: clinical trials performed on 94 negative samples showed 100 % specificity (interval 97 % -100 %, with a confidence level of 90 %).

Analysis by the CE-marked reference kit showed full match of results.

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 relative.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with *Neisseria 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; results should be interpreted with consideration of clinical and laboratory findings.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the *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 [9]. Further information can be found on the Robert-Koch-Institute website at: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Gonorrhoe.html.*

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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 box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR system, see paragraph 1
- DNA-Extraction Kit: RealLine DNA-Express , RealLine DNA-Extraction 2, RealLine DNA-Extraction 3, 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
- Safety laminar 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.

8. PREPARATION OF SPECIMEN

*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 recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure.
When using a kit of another supplier for the extraction of nucleic acids as recommended in p1., add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of 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 an extraction kit see chapter 1.2. 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 not more than 24 hours.
After initial opening shelf life of Positive Control sample at (2 - 8) °C is 1 month or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months.

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9. PROCEDURE

9.1 Preparation of the kit components:

Prior to the analysis test take the kit out of the refrigerator and keep the **Master Mix for PCR (MM)** closed in the package at (18 – 25) °C for at least 30 minutes. Then open the package and take the necessary number of tubes with MM (*including prepared samples and controls*). Each tube is intended for 10 tests.
Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.
After initial opening store MM at (2 – 8) °C for up to 3 months.

To prepare diluted Master Mix, add **300 µl** of Recovery Solution (RS) to each tube with MM. Mix gently, hold at room temperature for 15 minutes, and then carefully re-mix.
Store diluted MM at (2 – 8) °C for up to 7 days.
After initial opening shelf life of Recovery Solution at (2 – 8) °C is no more than 3 months.

9.2 Prepare an appropriate number of 0.2 ml tubes or a PCR plate. Label each tube for each specimen and control.
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 using a pipette tip with filter.

Add **25 µl** of corresponding extracted DNA solution to each tube using a new pipette tip with filter. Tightly close the tubes.

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

9.5 Program real time PCR system Program **Rotor-Gene Cyclers** as follows:

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

* measurement of fluorescent at 60 °C

For RealLine Cycler, iQ5 iCycler, CFX96, DT-96:

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

* measurement of fluorescent at 60 °C

9.6 Select the amplification detection channels:

- Collect data through **FAM** channel (RealLine Cycler, iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000) and ***Green*** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **IC DNA**;
- Collect data through **ROX** channel (RealLine Cycler, iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000), and ***Orange*** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of ***Neisseria gonorrhoeae*** **DNA**;

9.7 Program the position of the tubes with the specimens, PC and NC according to the Instruction Manual for the cycler in use.

9.8 Run the program.

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10. DATA ANALYSIS AND INTERPRETATION

10.1 The program should detect in **Positive Control** sample:

- increase of the IC DNA amplification signal along channel **FAM** / *Green* and determine the threshold cycle, IC **Ct**;
- increase of the *Neisseria gonorrhoeae* DNA amplification signal along channel **ROX** / *Orange* and determine the **Ct** value;

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

When **Ct** value for NC through **ROX** / *Orange* channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 9.9.).

10.3 For each sample the program should detect the increase of the amplification signal of IC DNA along 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 sample is considered **positive**, i.e. contains *Neisseria gonorrhoeae* DNA, when **Ct** value via **ROX** *(Orange)* channel for this sample is **less than or equals to 40.**

10.6 The sample is considered **negative** (not containing *Neisseria gonorrhoeae* DNA), if **Ct** value via **ROX** *(Orange)* channels for this sample is **above 40** or is not determined.

When 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.

10.7 If the **Ct** value for NC through the **ROX** / *Orange* channel **is less than or equal to 40,** it indicates the presence of contamination. In this case, all positive results of this individual PCR test 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 determined positive. Specimens that showed negative results in this run should be considered **negative**.

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 at the temperature up to 25 °C for no more than 10 days is acceptable.
- 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: store PC at (2 – 8) °C for no more than 1 month or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months
  Ready Master Mix (MM): unused not diluted MM at (2 - 8) °C not more than 3 months
  Diluted MM: at (2 - 8) °C for 2 weeks not more than 7 days
  Recovery Solution: at (2 - 8) °C for 3 months

12. REFERENCES

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ANNEX I: Settings for RealLine Cycler 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:

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).

Program real-time PCR cycler.

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

* measurement of fluorescent at 60 °C in FAM and ROX, (*Green* and *Orange*)

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 **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.

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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 *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.

Technical support: techsupport@bioron.de

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