Instructions for Use

RealLine Neisseria gonorrhoeae Str-Format

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

In vitro Diagnostics

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RealLine Neisseria gonorrhoeae (Str-Format)	**VBD4498**	**96 Tests**
valid from	**September 2019**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	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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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 (Str-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.

The Str-format Kit is intended for use with 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 **Str-Format Kit** contains 96 tubes (0.2 ml) in strips with lyophilized Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 96 tests, including control samples and the positive control sample.

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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

Universal Positive Control sample *(*PC)	1 vial, 1 ml
Ready Master Mix (RMM), lyophilized	96 test-tubes
The kit is additionally supplied with optical-transparent PCR-film	

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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.

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 %).

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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.*

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 outer box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED

- Real time PCR system, like described in paragraph 1
- DNA-Extraction Kit: RealLine DNA-Express , RealLine DNA-Extraction 2, RealLine DNA-Extraction 3, RealLine Extraction 100
- Internal Control reagent (VBC8881), if the kit is used with the extraction kits of other supplier;
- Negative Control Sample, if the kit is used with the extraction kits of other supplier;
- 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
- Razor or scalpel

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8. PREPARATION OF THE 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 strongly 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 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.

The assay is performed on extracted DNA specimens obtained from the clinical material using one of the DNA extraction kits listed in chapter1.2, according to the Instruction Manual to the kit.

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 no more than 24 hours, or like described in the Extraction manuals.
After initial opening of the tube, 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.

9. PROCEDURE

9.1 Preparation of the kit components

Prior to the test, take the kit out of the refrigerator and keep the **Ready Master Mix (RMM)** closed in the package at (18 – 25) °C for at least 30 min. Then open the package and cut off the necessary number of tubes with RMM (including the specimens and control samples) with the razor or scalpel. Cut the tubes together with the covering film.

Attention! *Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close with the clip.*
After initial opening the shelf life of RMM is 3 months at (2 – 8) °C.

9.2 Label the tubes with RMM for each specimen and control.
Attention! *Labels should be placed on the lateral side of the tubes.*

9.3 Add **50 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Tightly close the tubes with caps or seal with the PCR transparent film.

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

9.5 Program real time PCR system as follows:

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 real-time PCR data through the **FAM** channel for detection of amplification of IC DNA.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of *Neisseria gonorrhoeae* DNA.

9.7 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.

9.8 Run the program

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10. DATA ANALYSIS AND INTERPRETATION

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

- increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the *Neisseria gonorrhoeae* DNA amplification signal (channel **ROX**) and determine the **Ct** value;

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

When **Ct** value for NC through **ROX** channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 9.7.).

10.3 For each sample the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) 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 cycles 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 (**containing *Neisseria gonorrhoeae* DNA) when **Ct** value via **ROX** 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** channel 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 cycles, 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** 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 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: 1 month at (2 – 8) °C or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months.

Ready Master Mix (RMM): 3 months at (2 – 8) °C.

12. REFERENCES

1. Quillin S.J., Seifert H.S. *Neisseria gonorrhoeaea* host adaptation and pathogenesis. 2008. *Nat rev Microbiol*, 16(4) 226-240.
2. Lee J.S., Choi H.Y., Lee J.E., Lee S.H., Oum B.S. Gonococcal keratoconjunctivitis in adults. 2002. *Eye*, 16, 646-649.
3. Noble R.C., Cooper R.M., Miller B.R. Pharyngeal colonization by Neisseria gonorrhoeae and Neisseria meningitides in black and white patients attending a venereal disease clinic. 1979. *Br J Vener Dis*, 55, 14-19.
4. Danby C.S. et al. Patterns of extragenital chlamydia amd gonorrhea in women and men who have sex with men reporting a history of receptive and intercourse. 2016. *Sex Transm Dis*, 43, 105-109.
5. Little, J.W. Gonorrhea: update. 2006. *Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endodont*, 101, 137–143.
6. Sparling P.F., Handsfield H.H. *Neisseria gonorrhoeae*. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennet's Principles and Practice of Infectious Diseases*. 5th Ed. Philadelphia, PA: Churchill Livingstone, Inc., 2000, 2242-58.
7. Ross J.D. Systemic gonococcal infection.1996. *Genitourin Med*, 72(6), 404–407.
8. Sandstrom, I. Etiology and diagnosis of neonatal conjunctivitis. 1976. *Acta Paediatr Scand,* 76, 221–227.
9. Palmer H.M., et al. Evaluation of the Specificities of Five DNA Amplification Methods for the Detection of Neisseria gonorrhoeae. Feb 2003 Journal of Clinical Microbiology, 41 (2), 835-837

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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**
- **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.

Technical Support: techsupport@bioron.de

SPACE FOR YOUR NOTES:

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