Instructions for Use

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TRICHOMONAS VAGINALIS* AND *GARDNERELLA VAGINALIS* DNA BY REAL-TIME PCR METHOD IN ONE REACTION.

In vitro Diagnostics

CE

RealLine Trichomonas vaginalis / Gardnerella vaginalis (Str-Format)	**VBD0477**	**96 Tests**
valid from	**September 2023**	

BIORON Diagnostics GmbH
info@bioron.de - www.bioron.de

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

Explanation of symbols used in labelling

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

BIORON Diagnostics GmbH

In den Rauhweiden 20
67354 Römerberg
Germany

Phone +49 6232 298 44 0
Fax: +49 6232 298 44 29
info@bioron.de

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

Table of Content

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TRICHOMONAS VAGINALIS* DNA AND *GARDNERELLA VAGINALIS* DNA BY REAL-TIME PCR METHOD
In vitro Diagnostics

1. INTENDED USE

Clinical information

Trichomonas vaginalis - a flagellate - causes trichomoniasis, a disease that causes vaginal infections in women and urethritis in men. The transmission of trichomoniasis runs through sexual intercourse and is one of the most common sexually transmitted diseases.

Gardnerella vaginalis is a facultatively anaerobic Gram-variable rod that can cause bacterial vaginosis in some women as a result of a disruption in the normal vaginal microflora.

RealLine Trichomonas vaginalis / Gardnerella vaginalis (Str-format) assay kit is intended for the detection of *Trichomonas vaginalis* and *Gardnerella 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 extraction of DNA from clinical materials can be performed using the extraction kits:

- **RealLine DNA-Express (REF VBC8899-R)**
- **RealLine Extraction 100 (REF VBC8896-R)**
- **INSTANT Virus RNA/DNA Kit – IPC16 (Roboscreen GmbH, Germany)**
- **INSTANT Virus RNA/DNA Kit – FX (Roboscreen GmbH, Germany)**
- **VeriLab UMag (Laboveritas SIA)**

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

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

The kit is intended to use with block type cyclers: iQ™5 iCycler, CFX™96 (Bio-Rad, USA), DT-96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH)

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 followed strongly.

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

2. KIT CONTENTS

Universal Positive Control Sample (PC)	1 vial, 1 ml
Ready Master Mix (RMM), lyophilized	96 test-tubes 12 strips x 8 tubes
The kit is additionally supplied with optical-quality PCR-film	

3. PRINCIPLE OF THE METHOD

The 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-labelled 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.

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

4. SPECIFICATIONS

4.1. Sensitivity – the detection of 100 *Trichomonas vaginalis* DNA copies and 100 *Gardnerella vaginalis* DNA copies in Standard Reference Samples — 100%.

4.2. Specificity of *Trichomonas vaginalis* and *Gardnerella vaginalis* DNA detection using the Standard Reference Panel of negative samples is 100 %.

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

5. LIMITATIONS ODER 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 *Trichomonas vaginalis and Gardnerella vaginalis* 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 *Trichomonas vaginalis and Gardnerella vaginalis* 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.

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.
- 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 Kits, noted in p.1, with Internal control reagent
- Internal Control reagent (VBC8881) and Negative Control Sample, if the kit is used with the extraction kits of other supplier.
- 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;
- Scalpel.

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

8. PREPARATION OF SPECIMENS

*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 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 p.1, according to 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.

Store the extracted DNA at (2 – 8) °C for no more than 24 hours.
After initial opening, store PC at (2 – 8) °C for no more than 1 month or in 50 µl Aliquots at (-18 …-24) °C for no more than 3 months.

9. PROCEDURE

9.1. Preparation of the kit components:

Prior 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 minutes. Then open the package and cut the necessary number of tubes in strips with RMM (*including prepared samples and controls: 1 NC and 1 PC*) with the razor or scalpel. Cut the tubes together with the covering film.

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 at (2 – 8) °C is 3 months.

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. Carefully remove the covering film so that RMM remains in the tube.

9.4. Add **50 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 with caps or seal with the PCR transparent film. Start the PCR no later than 30 min after the DNA addition.
Attention! *The optical film should remain clean (free of any labels or marks).*

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

9.6. Program real time PCR system as follows:

Stage 1:	50°C	2min	
Stage 2:	95°C	2min	
Stage 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 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 *Trichomonas vaginalis* DNA.
- Collect real-time PCR data through the **HEX** channel for detection of amplification of *Gardnerella vaginalis* 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.

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

9.9. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

- For **Positive Control** the program should detect an increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**; an increase of the *Trichomonas vaginalis* DNA amplification signal (channel **ROX**) and determine the PC **Ct** value and an increase of the *Gardnerella vaginalis* DNA amplification signal (channel **HEX**) and determine the **Ct** value.

10.1 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 significant **ROX** or **HEX** fluorescent increase should appear (*no Gardnerella vaginalis or Trichomonas vaginalis DNA amplification*).

10.2 For each specimen the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**.

10.3 Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analysed 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.

10.4 The sample is considered **negative** (not containing *Trichomonas vaginalis and Gardnerella vaginalis DNA*), if **Ct** value via **ROX** is **above 40** and via **HEX** is **above 32** for this sample 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.

10.5 The sample is considered **positive**, i.e. contains *Gardnerella vaginalis* DNA, when **Ct** value via **HEX** channel for this sample is **less than or equals to 32.**

The sample is considered **positive**, i.e. contains *Trichomonas vaginalis* DNA, when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

10.6 If Ct value for NC through **HEX** channel is less than or equals to 32 and through **ROX** channel is less than or equals to 40, this indicates the presence of contamination. In case of contamination, 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 specimens of this run that were identified as 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 for the entire shelf life. The shelf life of the kit is 12 months from the manufacture date.
- Transport at (2 - 8) °C. Transportation at 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 (-18…-24) °C for 3 months.
Ready Master Mix (RMM):	3 months at (2 – 8) °C

Technical support: techsupport@bioron.de

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.

RealLine Trichomonas vaginalis / Gardnerella vaginalis Str-Format

BIORON Diagnostics GmbH
info@bioron.de - www.bioron.de