RealLine Candida albicans / Gardnerella vaginalis Fla-Format

Instructions for Use

RealLine Candida albicans / Gardnerella vaginalis Fla-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION AND DIFFERENTIATION OF *GARDNERELLA VAGINALIS* AND *CANDIDA ALBICANS* DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Candida albicans / Gardnerella vaginalis (Fla Format)	**VBD0445**	**100 Tests**
valid from	**September 2019**	

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

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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

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

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

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1. INTENDED USE

Clinical Information:
Candida albicans caused Candidiasis- this infection encompasses a range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. *Candida albicans* infections of the latter category are also referred to as candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant, and AIDS patients, as well as non-trauma emergency surgery patients.

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 Candida albicans / **Gardnerella vaginalis (Fla-format)** assay kit is designed to detect *Gardnerella vaginalis* and *Candida albicans vaginalis* DNA isolated from clinical specimens using the following extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

The **RealLine Candida albicans** / **Gardnerella vaginalis** assay kit is intended for the detection of *Candida albicans* and *Gardnerella vaginalis* DNA extracted from clinical specimens (epithelial cell swabs, urine, prostate fluid, semen) using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

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

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 the positive control samples.

The kit is intended for use with block-type PCR cyclers: iQ5 iCycler, CFX96 (Bio-Rad, USA), DT96 (DNA-Technology, Russia), RealLine Cycler (BIORON Diagnostics GmbH); and rotor-type PCR cyclers: Rotor-Gene 3000; Rotor-Gene 6000 (Corbett Research, Australia) and Rotor-Gene Q (Qiagen, Germany).

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
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. Sensitivity – the detection of 100 DNA copies of *Candida albicans* and *Gardnerella vaginalis* in samples – 100%.

4.2. Specificity of *Candida albicans* DNA 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. 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 *Candida albicans and/or 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 *Candida albicans and/or 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 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 pipettes and 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, like described in p.1
- DNA-Extraction Kit, RealLine DNA-Extraction 3 or see chapter1 Extractions Kits with Internal control reagent;
- 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.

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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 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.

The isolated DNA can be stored at (2 – 8) °C for 2 days.
After initial opening shelf life of Positive Control sample is 1 month at (2 – 8) °C or for 50 µl aliquots 3 month at (-18 … -60) °C

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 Soltion 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.

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.

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.

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For RealLine Cycler, iQ™ iCycler, iQ™5 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	

* Measure the fluorescence at 60°C

9.7 Select the amplification detection channels:

- Collect data through **FAM** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-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-96, Rotor-Gene 3000), or **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of ***Candida albicans***;

- Collect data through **HEX** channel (iQ5 iCycler, CFX96, RealLine Cyclers, DT-96, Rotor-Gene 3000), or **JOE/Yellow** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of ***Gardnerella vaginalis.***

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

9.9 Run the program.

10. DATA ANALYSIS AND INTERPRETATION

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

- Increase of the IC DNA amplification signal in channel **FAM** (*Green*) and determine the threshold cycle, IC **Ct**;
- Increase of the ***Candida albicans*** **DNA** amplification signal in channel **ROX** *(Orange)* and determine the **Ct** value;
- Increase of the ***Gardnerella vaginalis*** **DNA** amplification signal in channel **HEX** *(Yellow)* and determine the PC **Ct** value;

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA in channel **FAM** (*Green*) and determine the threshold cycle, IC **Ct**. No **ROX** *(Orange)* fluorescent increase should appear (*no Candida albicans or Gardnerella vaginalis DNA amplification*).

If **Ct** value for NC through **ROX** *(Orange)* channel **is less than or equal to 40,** or through **HEX** *(Yellow)* channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 10.7.).

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 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 **negative** (not containing *Candida albicans or Gardnerella vaginalis* DNA), if **Ct** value for this sample via **ROX** *(Orange)* channel is **above Ct 40** or via **HEX** *(Yellow)* channel is **above Ct 32** 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 required.

10.6 The sample is considered **positive** (containing ***Candida albicans*** DNA) when **Ct** value via **ROX** *(Orange)* channel for this sample is **less than or equals to 40.**

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

10.7 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 samples of this run that were identified as positive. Samples that showed negative results in this run should be considered as negative.

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11. STORAGE AND TRANSPORTATION

- Store 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 for 50 µl aliquots 3 month at (-18 … -60) °C*
  Ready Master Mix (MM): unused MM at (2 – 8) °C for up to 3 month.
  Diluted MM: at (2 – 8) °C for 7 days
  Recovery Solution: at (2 – 8) °C for 3 months.

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.

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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	
Step 2:	95°C	2min	
Step 3:	94°C	10 sec	50 cycles
	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**.
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.

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Results of *Gardnerella vaginalis* 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 *Candida albicans* 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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