GENTAUR

DATA SHEET

Bacterial Vaginosis Real-TM Quant

Multiplex real time pcr kit for quantitative detection of Gardnerella vaginalis, Atopobium vaginae, Lactobacillus spp. and total bacteriae quantity

HANDBOOK

for use with RotorGene™ 3000/6000/Q (Corbett Research, Qiagen), SmartCycler® (Cepheid), iQ iCycler™ and iQ5™ (Biorad), MX3000P® and MX3005P® (Stratagene), Applied Biosystems® 7500 Real Time PCR Systems (Applera)

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NAME

Bacterial Vaginosis Real-TM Quant

INTRODUCTION

Bacterial vaginosis (BV) is considered to be the most common cause of vaginal inflammation among both pregnant and non-pregnant women and prevalence between 4.9% and 36.0% have been reported from European and American studies. It previously was called nonspecific vaginitis or Gardnerella-associated vaginitis. The adult human vagina is a complex ecosystem containing an abundance of microorganisms. In women of childbearing age this system is dominated by ***Lactobacillus* spp.**, a genus of gram-positive, nonmotile rod-like bacteria, a defining characteristic of which is the ability to grow in acid media and tolerate acid conditions (pH < 4.5); lactobacilli also ferment carbohydrates to produce lactic acid and produce H2O2 which provides a natural defence against ***Gardnerella vaginalis***.

In bacterial vaginosis (BV) the balance of flora is changed with reduced numbers of lactobacilli (normal concentration $10^6 – 10^{10}$ CFU/ml) and an increase in numbers of other facultative and anaerobic species such as anaerobic cocci *Prevotella* spp., *Gardnerella vaginalis*, and *Mobiluncus* spp. (normal concentration < 10^3-10^5 CFU/ml). *G. vaginalis* is virtually always present at high concentrations in women who have BV but is also detected frequently in normal women and in some cases the concentration of *Gardnerella vaginalis* can reach 10^7-10^8 CFU/ml also in absence of BV, so the most important maker of BV is the ratio of logarithm concentration *Lactobacillus* spp and *G. vaginalis*.

The clinical significance of studying vaginal flora is that it helps determine the quantity of microorganisms and assess the ratio between the different groups of conditionally pathogenic microorganisms and the normal flora. The total quantity of bacteria serves as an indicator of infection level in the vaginal environment: under normal conditions it can vary between 10^6 and 10^9 (6-9 Log). The ratio between lactobacilli and the total bacterial quantity can be used as an indicator of the balance between the normal and conditionally pathogenic flora: the normal proportion of lactobacilli should be 95 to 100% of the total bacterial quantity.

INTENDED USE

Bacterial Vaginosis Real-TM Quant kit is for the quantitative detection of Gardnerella vaginalis, Atopobium vaginae, Lactobacillus spp. and total bacteriae quantity in the vaginal biotope. Bacterial Vaginosis Real-TM Quant kit allows to value the ratio among the total bacteriae quantity, lactobacilli and other facultative and anaerobic species (Gardnerella vaginalis, Atopobium vaginae) in the vaginal biotope; the total quantity of bacteriae is useful to value that the collected material is sufficient.

The logarithmic ratio between *Lactobacillus spp.* and the total quantity of bacteriae and also between *Lactobacillus spp.* and facultative pathogen microorganisms (*Gardnerella vaginalis* and *Atopobium vaginae*) allows to detect with high precision bacterial vaginosis disease related to normal vaginal microflora suppression (*Lactobacillus spp.)* and substitution with facultative pathogen microorganisms (*Gardnerella vaginalis* and *Atopobium vaginae*).

The use of **Bacterial Vaginosis Real-TM Quant** kit allows to monitor vaginal environment condition and to check the therapy efficiency.

PRINCIPLE OF ASSAY

Kit **Bacterial Vaginosis Real-TM Quant** is based on two major processes: isolation of DNA from specimens and Real Time amplification. DNA is extracted from the specimens, amplified in Real Time PCR and detected using fluorescent reporter dye probes specific for DNA *Gardnerella vaginalis,* DNA *Atopobium vaginae,* DNA *Lactobacillus spp.and DNA* Bacteriae.

MATERIALS PROVIDED

- PCR-mix-1-FRT, 1,2 ml;
- PCR-Buffer-FRT, 0,6 ml;
- TaqF Polymerase, 0,06 ml;
- Negative Control NC, 1,2 ml;*
- Pos Control BV+, 0,1 ml;*
- Pos Control BV-, 0,1 ml;*
- DNA-buffer (K-), 0,5 ml;
- Standards:
  - QS1 BK+, 0,2 ml;
  - QS2 BK+, 0,2 ml;

Contains reagents for 110 tests.

** must be used during the sample preparation procedure: add 100 µl of NC (Negative Control) to labeled Cneg; add 90 µl of NC (Negative Control) and 10 µl of BV+ control to the tubes labeled CposBV+; add 90 µl of NC (Negative Control) and 10 µl of BV- control to the tubes labeled CposBV-.*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA isolation kit
- Desktop microcentrifuge for "eppendorf" type tubes
- Vortex mixer
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Tube racks

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Use routine laboratory precautions. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas. Do not pipette by mouth.
3. Do not use a kit after its expiration date.
4. Do not mix reagents from different kits.
5. Dispose all specimens and unused reagents in accordance with local regulations.
6. Heparin has been shown to inhibit reaction. The use of heparinized specimens is not recommended.
7. Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
8. Once the reagents have been thawed, vortex and centrifuge briefly the tubes.
9. Prepare quickly the Reaction mix.
10. Specimens may be infectious. Use Universal Precautions when performing the assay.
11. Specimens and controls should be prepared in a laminar flow hood.
12. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
13. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant. Follow by wiping down the surface with 70% ethanol.
14. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
15. Material Safety Data Sheets (MSDS) are available on request.
16. Use of this product should be limited to personnel trained in the techniques of amplification.
17. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification Area. Do not return samples, equipment and reagents in the area where you performed previous step. Personnel should be using proper anti-contamination safeguards when moving between areas.

STORAGE INSTRUCTIONS

Bacterial Vaginosis Real-TM Quant must be stored at -20°C. The **Bacterial Vaginosis Real-TM Quant** kit can be shipped at 2-8°C but should be stored at -20°C immediately on receipt.

STABILITY

Bacterial Vaginosis Real-TM Quant is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

QUALITY CONTROL

The complete kit has been tested on an RotorGene 6000 (Corbett Research).
Certificates of Analyses are available on request at info@sacace.com.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Bacterial Vaginosis Real-TM Quant SC can analyze DNA extracted from:

- *vaginal swabs:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec. Snap off shaft at scored line, leaving end inside tube.

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C.
Before DNA extraction it is necessary to defreeze the samples (if stored for long time), to vortex them vigorously and centrifuge for 3-5 sec to remove drops from the cap.
Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kit is recommended:

⇒ **DNA-Sorb-A** (Sacace, REF K-1-1/A).

Please carry out the DNA extraction according to the manufacturer's instructions.

PROTOCOL:

1. Prepare required quantity of tubes or PCR plate.
2. Prepare for each sample in the new sterile tube **10*N µl** of **PCR-mix-1**, **5*N µl** of **PCR-mix-2 buffer** and **0,5*N** of **Hot Start DNA Polymerase.**
3. Add **15 µl** of **Reaction Mix** into each tube.
4. Add **10 µl** of **extracted DNA** sample to appropriate tube with Reaction Mix.
5. Prepare for each run 2 standards and 1 neg control:
   - add **10 µl** of **DNA-buffer** to the tube labeled C*neg*;
   - for quantitative analysis prepare 4 tubes and perform QS1 and QS2 standards twice: add **10 µl** of **QS1** and **QS2** into labeled tubes;

Close tubes and transfer them into the instrument in this order: samples, negative controls, positive control, Standards

6. Insert the tubes in the thermalcycler and program the instruments as indicated below

Program the Real Time PCR instrument according to Table*.

	Rotor and plate type instruments[1]		
Step	Temperature, °C	Time	Cycles
1	95	15 min	1
2	95	5 s	5
	60	20 s	
	72	15 s	
3	95	5 s	40
	60	30 s Fluorescence detection on FAM/Green, JOE/Yellow/Hex ROX/Orange/TexasRed Cy5/Red	
	72	15 s	

[1] For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen), iQ5™/iQ iCycler™ (BioRad); Mx3000P/ Mx3005P™ (Stratagene), Applied Biosystems® 7500 Real Time PCR (Applera)

RESULTS INTERPRETATION:

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.

Gardnerella vaginalis is detected on the FAM/Green channel

Atopobium vaginae on the JOE/Yellow/HEX/Cy3 channel

Lactobacillus spp on the ROX/Orange/TexasRed channel

Total bacteriae DNA on the Cy5/Red channel.

Table 2. Results for controls

Control	Stage for control	Channels, copies/reaction			
		FAM/Green	JOE/Yellow	ROX/Orange	Cy5/Red
NC	DNA isolation	<5	<5	<50	<5000
BV-	DNA isolation	<5	<5	$>10^6$	$>10^6$
BV+	DNA isolation	$>10^4$	$>10^4$	$<10^4$	$>10^6$
K-	PCR	<5	<5	<5	<500

Interpretation of results

Coefficient KC1 is obtained by the logarithmic difference between *Lactobacillus* spp. (Lac) concentration and the total quantity of bacteriae (Bac):

$$KC1 = \lg(K_{Lac}*100) - \lg(K_{Bac}*100),$$

where K_{Lac} = **copies Lac/reaction (ROX/Orange/TexasRed channel),**
K_{Bac} = **copies Bac/reaction (Cy5/Red channel)**

Coefficient KC2 is obtained by the logarithmic difference between *Lactobacillus* spp. (Lac) concentration and the total quantity of anaerobic microorganisms (*Gardnerella vaginalis+Atopobium vaginae*):

$$KC2 = \lg(K_{Lac}*100) - \lg[(K_{Gv}*100)+(K_{Av}*100)],$$

where K_{Gv} = **copies Gv/reaction (FAM/Green channel)**
K_{Av} = **copies Av/reaction (JOE/Yellow/HEX/Cy3 channel)**
K_{Lac} = **copies Lac/reaction (ROX/Orange/TexasRed channel)**

Note: *K *100 = copies/ml*

Basing on the values of KC1 and KC2 the following diagnosis can be obtained:

– **Grade 1 Normal vaginal flora** ($-1 \leq KC1 < 1$; $KC2 \geq 1$) – the quantity of *Lactobacillus* spp. matches with the quantity of bacteriae. *Gardnerella vaginalis* and/or *Atopobium vaginae* are absent or much lower than *Lactobacillus* spp. (*Lactobacillus* spp is predominant);

– **Grade 2 Intermediate vaginal flora** ($-2 \leq KC1 < 1$; $KC2 < 1$) – the quantity of *Lactobacillus* spp. is lower than the quantity of bacteriae, the quantity of *Gardnerella vaginalis / Atopobium vaginae* is higher than *Lactobacillus* spp. (Mixed flora with some *Lactobacillus* spp. present, but *Gardnerella vaginalis / Atopobium vaginae* also present);

– **Grade 3 Bacterial vaginosis** ($KC1 < -2$, $KC2 < 1$) – the quantity of *Lactobacillus* spp. is much lower than the quantity of bacteriae, the quantity of *Gardnerella vaginalis* and/or *Atopobium vaginae is* much higher than *Lactobacillus* spp (few or absent *Lactobacillus* spp., predominantly *Gardnerella vaginalis* and/or *Atopobium vaginae*).

– **Grade 4 Change in vaginal flora composition with unknown prevalent (bacterial) species** ($KC1 < -1$; $KC2 \geq 1$) – the quantity of *Lactobacillus* spp is lower than the quantity of bacteriae, *Gardnerella vaginalis* and/or *Atopobium vaginae* are absent or much lower than *Lactobacillus* spp. (*Lactobacillus* spp., *Gardnerella vaginalis*, *Atopobium vaginae* are absent or few; predominantly other bacterial species, for example *E. Coli*, *Proteus* etc);

TROUBLESHOOTING

1. Calculated values (copies/reaction) > 5 for Negative Control of Extraction and/or Negative Control of PCR in FAM/Green channel and/or Joe/Yellow channel.
    - Contamination. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive controls at last.
        - ⇒ Repeat the experiment retesting only the samples positive for *Gardnerella vaginalis* and/or *Atopobium vaginae*.
        - ⇒ Repeat the PCR preparation with the new set of reagents.
2. Variation of more than 30% in calculated values (copies/reaction) compared to values reported in the Data Card for any calibrator (QS1 or QS2).
    - Check correct position of calibrator tubes.
    - Check correct insertion of concentration values for calibrators.
3. Correlation coefficient of standard curve < 0.90.
    - Repeat the experiment.
    - Check correct insertion of concentration values for calibrators.
4. No Ct results for BV- control on ROX/Orange/TexasRed channel or Cy5/Red channel.
    - Control did not work, repeat experiment.
5. No Ct results for BV+ control on any channel.
    - Control did not work, repeat experiment.
6. Samples with concentration of *Lactobacillus* spp. or total bacteria $<10^6$ copies/ml ($<10^4$ copies/reaction) are considered invalid because of low amount of material. This situation can occur if:
    - Starting material was insufficient or wrongly collected.
    - Sample is collected from women patient under antibiotics treatment: in this case we suggest to repeat the sampling 2 weeks after the end of treatment.
    - Sample is collected from young women in pre-pubertal phase.
    - Sample is collected from women in menopause.
7. Calculated total quantity of bacteria is lower than *Lactobacillus* spp.
    - Experiment results are invalid.
    - Repeat experiment starting from nucleic acid extraction.
    - If necessary, repeat experiment starting from sample collection.

EXPLANATION OF SYMBOLS

Catalogue Number

For *Research Use Only*

Lot Number

Expiration Date

Contains reagents

Caution!

Version

Manufacturer

Temperature limitation

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium