IVD For *in Vitro* Diagnostic Use

CE

Bacterial Vaginosis Real-TM Quant

HANDBOOK

Multiplex Real Time PCR kit for quantitative detection of Gardnerella vaginalis, Atopobium vaginae, Lactobacillus spp. and total bacteria quantity

REF B74-100FRT

REF TB74-100FRT

Σ 100

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 H_2O_2 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 bacteria. Today more than 200 BV-associated microorganisms are described (mostly anaerobic). The studies in the recent years show that *Gardnerella vaginalis* plays the key role in development of bacterial vaginosis because of several reasons:

– it is detected almost in all patients with bacterial vaginosis,
– it has the strongest pathogenic potential among all the described BV-assosiated bacteria,
– it has significant adhesiveness,
– it forms biofilm,
– it produces cytolisin (vaginolisin), sialidase.

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*.
Another marker of bacterial vaginosis is *Atopobium vaginae.* It is a highly specific microorganism for this syndrome. *Atopobium vaginae* is a highly metronidazole resistant microorganism. Thus, its detection can cause the change of treatment strategy.

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.

INTENDED USE

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

The logarithmic ratio between *Lactobacillus spp.* and the total quantity of bacteria 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* of Bacteria.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B74-100FRT)

Part N° 2 – **"Bacterial Vaginosis Real-TM Quant":** Real Time amplification

- **PCR-mix-1-FRT**, 1,2 ml;
- **PCR-mix-2**, 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+**, 0,4 ml;
  - **QS2 BK+**, 0,4 ml;

Contains reagents for 110 tests.

Module No.2: Complete Real Time PCR test with DNA purification kit (TB74-100FRT)

Part N° 1 – **"DNA-Sorb-A":** Sample preparation;

- **Lysis Solution**, 2 x 15 ml;
- **Sorbent**, 2 x 1,0 ml;
- **Washing Solution**, 2 x 50 ml;
- **DNA-eluent**, 2 x 5 ml;
- **Transport medium**, 30 ml.

Contains reagents for 100 tests.

Part N° 2 – **"Bacterial Vaginosis Real-TM Quant":** Real Time amplification

- **PCR-mix-1-FRT**, 1,2 ml;
- **PCR-mix-2**, 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+**, 0,4 ml;
  - **QS2 BK+**, 0,4 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 Cpos BV+; add 90 µl of NC (Negative Control) and 10 µl of BV- control to the tubes labeled Cpos BV-.*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- DNA extraction kit (Module No. 1)
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Biohazard waste container
- Refrigerator
- Freezer

Zone 2: Real Time amplification:

- Real Time Thermal cycler
- Reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Vortex mixer
- Freezer, refrigerator

STORAGE INSTRUCTIONS

Bacterial Vaginosis Real-TM Quant must be stored at -20°C. **DNA-sorb-A** must be stored at 2-8°C. The kits can be shipped at 2-8°C but should be stored at 2-8°C and -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

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD **_In Vitro_ Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- ⚠ Lysis Solution contains guanidine thiocyanate*. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

**** Only for Module No.2***

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Bacterial Vaginosis Real-TM Quant 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.

***** unreliable results can occur if sampling is not performed correctly, in cases like:

- insufficient material quantity or improper sampling;
- the material was obtained from another locus (for example, from cervical canal);
- patient under antibiotic treatment. In this case, it is recommended to perform the analysis 2 weeks after the end of therapy;
- the material was obtained from prepubertal girls (before the menarche) or menopausal women;

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

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

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

⇒ **SaMag STD DNA Extraction kit** (Sacace, REF SM007).

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

SPECIMEN AND REAGENT PREPARATION (reagents supplied with the module no.2)

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60–65°C until disappearance of ice crystals. Prepare required quantity of 1.5 ml polypropylene tubes including one tube for **Negative Control of Extraction**.
2. Add to each tube **300 µl** of **Lysis Solution**.
3. Add **100 µl** of **Samples** to the appropriate tube.
4. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Neg Control** provided with the amplification kit) to the tube labeled C*neg*.
   - add **90 µl** of **Negative Control** and **10 µl** of **BV+ control** to the tubes labeled *Cpos BV+;*
   - add **90 µl** of **Negative Control** and **10 µl** of **BV- control** to the tubes labeled *Cpos BV-.*
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 5-7 sec. If the sample is not completely dissolved it is recommended to re-centrifuge the tube for 5 min at a maximum speed (12000-16000 g.) and transfer the supernatant into a new tube for DNA extraction.
6. Vortex vigorously **Sorbent** and add **20 µl** to each tube.
7. Vortex for 5-7 sec and incubate all tubes for 3 min at room temperature. Repeat this step.
8. Centrifuge all tubes for 30 sec at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
9. Add **500 µl** of **Washing Solution** to each tube. Vortex very vigorously and centrifuge for 30 sec at 10000g. Remove and discard supernatant from each tube.
10. Repeat step 9 and incubate all tubes with open cap for 5-10 min at 65°C.
11. Resuspend the pellet in **100 µl of DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
12. Centrifuge the tubes for 1 min at 12000g.
13. The supernatant contains DNA ready for amplification. If amplification is not performed in the same day of extraction, the processed samples can be stored at 2-8°C for at maximum period of 5 days or frozen at –20°/-80°C.

PROTOCOL (total vol:25 µl)

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 FRT**, **5*N µl** of **PCR-mix-2** and **0,5*N** of **TaqF 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 labelled 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

Amplification

1. Create a temperature profile on your instrument as follows:

	Rotor-type Instruments[1]			**Plate- or modular type Instruments[2]**		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] *For example, Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, SaCycler-96™ (Sacace),CFX96/ iQ5™/iQ iCycler™ (BioRad); Mx3000P/ Mx3005P™ (Stratagene), Applied Biosystems® 7500 Real Time PCR (Applera)*

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green, JOE/Yellow/Hex/Cy3, ROX/Texas Red and Cy5/Red fluorescence channels.

INSTRUMENT SETTINGS

Rotor-type instruments

Channel	*Calibrate / Gain Optimisation…*	*Threshold*	*More Settings/ Outlier Removal*	*Slope Correct*
FAM/Green	*from 5 to 10 FL*	*0.05*	*10-20 %*	*On*
JOE/Yellow	*from 5 to 10 FL*	*0.05*	*10-20 %*	*On*
ROX/Orange	*from 5 to 10 FL*	*0.05*	*5-10 %*	*On*
Cy5/Red	*from 5 to 10 FL*	*0.05*	*10-30 %*	*On*

Plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

Boundary concentration values, copies/ml (refer to DataCard supplied with kit)

Sample	FAM/Green	JOE/Yellow	ROX/Orange	Cy5/Red
C–	< boundary	< boundary	< boundary	< boundary
NCA	< boundary	< boundary	< boundary	< boundary
BV–	< boundary	< boundary	> boundary	> boundary
BV+	> boundary	> boundary	< boundary	> boundary

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 **is detected on the JOE/Yellow/HEX/Cy3 channel**

Lactobacillus spp **is detected on the ROX/Orange/TexasRed channel**

Total bacteriae DNA is detected on the Cy5/Red channel.

Principle of interpretation is the following:

- the result of a sample is considered **positive** in the channels for the **FAM, JOE, ROX** and **Cy5** if fluorescence curve is S-shaped and crosses the threshold line.
- the result of a sample is considered **negative** in the channels for the **FAM, JOE, ROX** and **Cy5** if fluorescence curve does not cross the threshold line (*Ct* value is absent) and does not have typical S-shape.
- the result of a sample is considered **unreliable** if the signal in the channel for the **Cy5** is absent or when ***Calc Conc*** value of an analyzed sample in the channel for the **Cy5** is less than 1000 copies/reaction (that corresponds to 10^5 copies/ml).
- the result of a sample is considered **invalid** if the signal in the channel for the **Cy5** is absent (no *Ct* value) or if ***Calc Conc*** value of an analyzed in the channel for the **ROX** is **greater** than ***Calc Conc*** value in the channel for the **Cy5** by more than **0.5 log.**

Interpretation of results

The concentrations of the DNA-calibrators needed for the calculation of the samples and controls concentrations are specified in the *Data Card* supplied with each PCR kit.

The results analysis is performed using the Microsoft Excel software according to the enclosed instruction. See the calculation algorithm of the software below.

For obtaining the results it is necessary to:

- Enter the values from the *Data Card* to the calculation table*;*
- Complete the columns in the ***Run Information*** section;
- Copy the sample names and the *Ct* values for all the channels;
- Click the ***Calculate*** button. The following data will be displayed automatically in the corresponding cells:
    1. The values of ratio coefficients (KC1, KC2 and KC3),
    2. Status of the samples,
    3. The results for each sample and its interpretation.

Then obtained results and the interpretation should be copied to the laboratory form. The reference values should be specified for each test parameter. The reference value for total amount of Bacteria DNA is ≥ 10^6 copies/ml. The less amount of Bacteria DNA in the sample can be obtained as a result of improper sampling, analysis of the material from girls before the menarche or menopausal women, antibiotic/antiseptic use or syringing during two weeks prior to sampling. Healthy woman has almost the same concentration of *Lactobacillus* spp. DNA and Bacterial DNA because the normal vaginal flora is dominated by *Lactobacillus* spp. The *Gardnerella vaginalis* and *Atopobium vaginae* can be present on the vaginal mucosa in low concentrations not exceeding the concentration of *Lactobacillus* spp. DNA.

Calculation algorithm

Microsoft Excel software calculates ratio coefficients KC1, KC2 and KC3.

KC1 ratio coefficient represents the relative concentrations of the *Lactobacillus* spp. (Lac) and anaerobic microorganisms (*Gardnerella vaginalis+Atopobium vaginae* (Gv+Av)). It is calculated as the difference between logarithms of DNA concentrations of specified microorganisms:

KC1 = log(Lac DNA) – log(Gv+Av DNA),

KC2 ratio coefficient represents the relative concentrations of the total amount of bacteria (Bac) and *Lactobacillus* spp. (Lac). It is calculated as the difference between logarithms of DNA concentrations of specified microorganisms:

KC2 = log(Bac DNA) – log(Lac DNA),

KC3 ratio coefficient represents the relative concentrations of the total amount of bacteria (Bac) and anaerobic microorganisms (*Gardnerella vaginalis+Atopobium vaginae* (Gv+Av)). It is calculated as the difference between logarithms of DNA concentrations of specified microorganisms:

$$\mathbf{KC3 = log(Bac\ DNA) - log(Gv+Av\ DNA)},$$

The following results are displayed automatically on the basis of KC1, KC2 and KC3:

1) ***Relative concentrations of microbial DNA correspond to bacterial vaginosis*** (KC1<0.5);
2) ***Relative concentrations of microbial DNA do not correspond to bacterial vaginosis*** (KC1>1);
3) ***Relative concentrations of microbial DNA correspond to intermediate flora*** (0,5≤KC1≤1);
4) ***Relative concentrations of microbial DNA correspond to flora alteration, unspecified etiology*** (KC2>1, KC3>2, any KC1 value);
5) ***Bacterial load decreased*** (KC1>1 so the sample is considered BV negative and total amount of Bacteria DNA is less than 10^6 copies/ml but greater than 10^5 copies/);
6) ***Bacterial load insufficient for analysis*** (total amount of Bacteria DNA is less than 10^5 copies/ml).

Examples of results

1. The amount of *G.vaginalis* and/or *A.vaginae* is almost equal or exceeds the amount of *Lactobacillus* spp. – ***Relative concentrations of microbial DNA correspond to bacterial vaginosis***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$3*10^7$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$3*10^4$**	is not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	**$2*10^7$**	is not greater than *Lactobacillus DNA* concentration
Atopobium vaginae DNA (copies/ml)	**$3*10^6$**	is not greater than *Lactobacillus DNA* concentration
Result	***Relative concentrations of microbial DNA correspond to bacterial vaginosis***	
Note – It should be taken into account for therapy that *Atopobium vaginae* is highly metronidazole resistant microorganism * * This note is important for giving the results because the detection of *Atopobium vaginae* in the clinical material can cause the change of therapy strategy.		

2. The *G.vaginalis* and/or *A.vaginae* are absent or its amount is substantially less than the *Lactobacillus* spp. amount – ***Relative concentrations of microbial DNA do not correspond to bacterial vaginosis***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$1*10^7$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$1*10^7$**	is not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration
Atopobium vaginae DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration

3. The amount of *G.vaginalis* and/or *A.vaginae* is similar to the amount of *Lactobacillus* spp. but doesn't exceed the limit value – ***Relative concentrations of microbial DNA correspond to intermediate flora***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$8*10^6$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$8*10^6$**	is not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	**$2*10^6$**	is not greater than *Lactobacillus DNA* concentration
Atopobium vaginae DNA (copies/ml)	**$2*10^4$**	is not greater than *Lactobacillus DNA* concentration
Result	***Relative concentrations of microbial DNA correspond to intermediate flora***	

4. The amount of *Lactobacillus* spp. is reduced relative to the total amount of bacteria, whereas *G.vaginalis* and/or *A.vaginae* are absent or its amount is substantially less than total amount of bacteria – ***Relative concentrations of microbial DNA correspond to flora alteration, uspecified***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$1*10^7$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$4*10^3$**	is not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration
Atopobium vaginae DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration
Result	***Relative concentrations of microbial DNA correspond to flora alteration, uspecified***	

5. *G.vaginalis* and/or *A.vaginae* are absent or its amount is substantially less than *Lactobacillus* spp. amount, total amount of bacteria DNA is less than 10^6 copies/ml and more than 10^5 copies/ml – ***Bacterial load decreased (BV negative)***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$9*10^5$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$8*10^5$**	is not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration
Atopobium vaginae DNA (copies/ml)	is not detected	is not greater than *Lactobacillus DNA* concentration
Result	***Bacterial load decreased***	

6. Total amount of bacteria DNA is less than 10^5 copies/ml – ***Bacterial load insufficient for analysis***.

Parameters	Results	Reference value
Bacterial DNA – total amount of bacteria (copies/ml)	**$7*10^4$**	≥ 10^6
Lactobacillus spp. DNA (copies/ml)	**$1*10^3$**	Not less than Bacterial DNA
Gardnerella vaginalis DNA (copies/ml)	is not detected	Not more than concentration of *Lactobacillus DNA*
Atopobium vaginae DNA (copies/ml)	is not detected	Not more than concentration of *Lactobacillus DNA*
Result	***Bacterial load insufficient for analysis***	

SPECIFICATION

Sensitivity

The analytical sensitivity of **Bacterial Vaginosis Real-TM Quant** PCR kit is the following:

Clinical material	DNA extraction kit	Sensitivity, copies/ml
Vaginal swabs	DNA-sorb-A	$2.5x10^3$

Specificity

PCR kit detects *Gardnerella vaginalis, Atopobium vaginae, Lactobacillus* spp. DNA and bacterial DNA. The clinical specificity of the kit is proved by the clinical material examination with the following results confirmation by the sequence analysis of the amplification fragments.

Nonspecific reactions were absent during testing of human DNA samples and DNA panels of the following microorganisms: *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Candida glabrata, Candida krusei, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria* spp., *Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV*-1 and *HSV*-2, *CMV*, and *HPV.*

TROUBLESHOOTING

1. If **Calc Conc** value greater than 5 copies/reaction (that corresponds to 500 copies/ml) appears in the results grid for the Negative control of extraction (C–) and/or Negative control of amplification (NCA) in the FAM/Green and/or JOE/HEX/Yellow channel, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. The analysis should be repeated from the extraction stage for all samples in which *Gardnerella vaginalis* and/or *Atopobium vaginae* DNA was detected. The measures for detection and elimination of contamination source should be assumed.
2. If the values (copies/reaction) of QS1 and QS2 calibrators differ from the specified ones by more than 30 %, check the tubes order in the instrument. For rotor-type instruments well 1 must be filled with any tube containing reaction mix.
3. If the value of the correlation coefficient, R^2, is less than 0.9, calibration failure has occurred. Make sure that calibrators are set correctly and correct if necessary. If it does not help, repeat PCR for all samples and calibrators.
4. If the *Ct* value of the Positive control of extraction (BV–) is absent in the ROX/Orange or Cy5/Red channels, the results of analysis are considered invalid for all samples. Repeat PCR for all the samples.
5. If the *Ct* value of the Positive control of extraction (BV+) is absent in one or more channels (FAM/Green, JOE/HEX/Yellow, ROX/Orange, or Cy5/Red), the results of analysis are considered invalid for all samples. Repeat PCR for all the samples.
6. If a signal of a test sample is absent in the Cy5/Red channel or if **Calc Conc** value in the Cy5/Red channel is less than 10^5 copies/ml (1000 copies/reaction), the result is considered unreliable and PCR should be repeated for this sample. If the same result is reproduced, re-sampling is recommended.
7. If the signal of a sample is absent (*Ct* value is absent) in the Cy5/Red channel or if the quantity of *Lactobacillus* spp. is greater than the total quantity of bacteria **by more than 0.5 Log**, the result for this sample is considered **invalid**. The analysis of the sample should be repeated starting from the extraction stage. If the same result is reproduced, re-sampling is recommended.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX96/iCycler and iQ5 are trademarks of Bio-Rad Laboratories®
* The Rotor-Gene™ Technology is a registered trademark of Qiagen®
* Mx3000P/ Mx3005P™ is a registered trademark of Stratagene®
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