Instruction for Use

diarellaBV-1

real time PCR Kit

For *in vitro* detection and quantification of the DNA of Gardnerella vaginalis, Atopobium vaginae and Lactobacillus species extracted from biological specimens.

REF **G01146-96**

Σ 96

- 18°C

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1 Intended Purpose

The Kit is designed for the quantitative detection of the nucleic acid of Gardnerella vaginalis, Atopobium vaginae and Lactobacillus species in eluates from biological specimens. The assay is an in vitro diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform. The assay serves as an aid in the diagnosis, screening and monitoring of bacterial vaginosis.

2 Pathogen Information

Gardnerella vaginalis is a facultative anaerobic, coccoid bacterium of the family of Bifidobacteriaceae. In low copy numbers it is part of the common vaginal flora. When the equilibrium of the vaginal microbiome is disturbed, the number of G. vaginalis can increase a lot. G. vaginalis is not considered to be the cause of bacterial vaginoses [1] [2] but it can be used as a reporter for the altered vaginal microbiome.

Atopobium vaginae is a facultative anaerobic, rod-shaped bacterium of the family of Atopobiaceae. It is also known as Fannyhessea vaginae and isolated in a lot of cases of bacterial vaginosis [3]. A. vaginae also seems to be implicated in treatment failures and birth risks.

Lactobacilli, namely L. crispatus, L. gasseri, L. jenseni and L. iners are rod-shaped aerotolerant anaerobes and represent the dominant bacteria clade in the human vaginal microbiome. In cases of bacterial vaginosis, the number of lactobacilli is often declined and the equilibrium in the vaginal microbiome disturbed. This results usually in a loss of acidity.

3 Principle of the Test

The diarellaBV-1 real time PCR Kit contains specific primers and dual-labeled probes for the amplification of the DNA of Gardnerella vaginalis, Atopobium vaginae and Lactobacillus species extracted from biological specimens.

The presence of nucleic acid is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the Lactobacillus species specific probes is measured in the FAM channel. The fluorescence of the Gardnerella vaginalis specific probes is measured in the ROX channel. The fluorescence of the Atopobium vaginae specific probes is measured in the Cy5 channel.

The simple presence of the Gardnerella vaginalis or Atopobium vaginae does not compulsory imply a bacterial vaginosis. The diagnosis is dependent on the proportions of the different bacteria in relation to each other.

For the quantification of the different bacteria, the diarellaBV-1 real time PCR Kit contains three quantification standards. Each quantification standard includes synthetic DNA of all of the three individual target sequences.
Furthermore, diarellaBV-1 real time PCR Kit contains a Control DNA (Internal Process Control, IPC), which is added during DNA extraction and detected in the same reaction by a HEX-labeled probe.
The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of the diarellaBV-1 real time PCR Kit

Label	Lid Colour	Content 96	Concentration copies/µl
Reaction Mix	yellow	1 x 1344 µl	-
Positive Control	red	1 x 150 µl	-
Negative Control	green	1 x 150 µl	-
Control DNA	colourless	1 x 480 µl	-
Standard 1	black	1 x 100 µl	100,000
Standard 2	violett	1 x 100 µl	10,000
Standard 3	orange	1 x 100 µl	1,000

5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

The diarellaBV-1 real time PCR Kit is shipped on dry ice. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible. Protect kit components from direct sunlight during the complete test run.

7 Warnings and Precautions

Read the Instruction for Use carefully before using the product.
Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.
- Do not mix components from different lots.

8 Sample Material

Starting material for diarellaBV-1 real time PCR is DNA isolated from biological specimens. Common samples for the DNA extraction for the analysis of the vaginal microbiome are vaginal swabs or urine.

9 Sample Preparation

Commercial kits for DNA isolation such as the following are recommended:

- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Please follow the Instruction for Use of the respective extraction kit.

Important:
In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.
Comparing the amplification of the Control DNA in the samples to the amplification of the Internal Control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

Please note the chapter ‚Control DNA'.
If the real time PCR is not performed immediately, store extracted DNA according to the instructions given by the manufacturer.

10 Control DNA

A Control DNA is supplied as extraction control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.

Add 5 µl Control DNA per extraction. Mix well. Perform the DNA isolation according to the manufacturer's instructions.

The Control DNA must be added to the Lysis Buffer of the extraction kit.

11 Real time PCR

11.1 Important Points Before Starting:

- Please pay attention to chapter 7 ‚Warnings and Precautions'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.

- In every PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed, and centrifuged very briefly.

11.2 Quantification Standards

The diarellaBV-1 real time PCR Kit includes three Quantification Standards. Use 6 µl of each Quantification Standard to evaluate the target concentrations of the PCR targets. The concentrations are calculated according to chapter 'Data Analysis'.
The Quantification Standards can be used in every single experiment or saved in a standard curve file on the qPCR instrument. It is recommended to produce a new Quantification Standard file for each new lot.

11.3 Procedure

The Master Mix contains all of the components needed for the real time PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2:Preparation of the Master Mix

Volume per Reaction	Volume Master Mix
14.0 µl Reaction Mix	14.0 µl x (N+1)

Real time PCR set-up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control, the Negative Control and the Standards (optional) to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 3).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the real time PCR

Component	Volume
Master Mix	14.0 µl
Sample	6.0 µl
Total Volume	20.0 µl

11.4 Instrument Settings

For the real time PCR use the thermal profile shown in Table 4.

Table 4: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

If in the same run samples should be tested for pathogens with RNA genome, use the thermal profile shown in Table 5.

Table 5: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing and Extension	40 sec	60°C	
	Acquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to
Table 6.

Table 6: Overview of the instrument settings required for the diarellaBV-1 real time PCR.

Real time PCR Instrument	**Parameter**	**Detection Channel**	**Notes**		
LightCycler 480II			Color Compensation Kit Multiplex 1 (G070MP1-CC) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	Lactobacillus spp.	465-510	1	10	1
	Control DNA (IPC)	533-580	1	10	2
	Gardnerella vaginalis	533-610	1	10	2
	Atopobium vaginae	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	Lactobacillus spp.	FAM	Gain 8	Reference Dye: None	
	Control DNA (IPC)	HEX	Gain 1		
	Gardnerella vaginalis	ROX	Gain 1		
	Atopobium vaginae	Cy5	Gain 4		
QuantStudio 5 CFX96 CFX Opus 96 Aria Mx qTower³ G	Lactobacillus spp.	FAM	Option Reference Dye ROX: NO		
	Control DNA (IPC)	HEX			
	Gardnerella vaginalis	ROX			
	Atopobium vaginae	Cy5			
Mic qPCR Cycler	Lactobacillus spp.	Green	Gain 8		
	Control DNA (IPC)	Yellow	Gain 10		
	Gardnerella vaginalis	Orange	Gain 10		
	Atopobium vaginae	Red	Gain 10		

12 Data Analysis

Following results can occur:

Signal/C_T Values				
FAM Channel Lactobacillus spp.	**Cy5 Channel** Atopobium vaginae	**ROX Channel** Gardnerella vaginalis	**HEX Channel** IPC	**Interpretation**
positive (one or more parameters)			positive or negative[1]	**For Interpretation, comparison of the calculated quantities is needed.**
negative	negative	negative	≤ 34 [2]	**Negative result**, the sample contains no Lactobacillus spp., A. vaginae or G. vaginalis DNA**.**
negative	negative	negative	negative or > 34 [2]	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred during DNA extraction.

[1] A strong positive signal in the FAM, ROX or the Cy5 channel can inhibit the IPC. In such cases the result for the Control DNA can be neglected.

[2] In case of high C_T values, the IPC should be compared to the water extraction control as described in the chapter 'Assay validation'.

Quantitative Analysis

The diarellaBV-1 real time PCR Kit includes three Quantification Standards. The calculation of the bacterial load can be done manually or, in most PCR cyclers, directly in the instrument software by the definition of quantification standards. The result will show the concentration of the specific bacterial load in the eluate.

$$C_T = a * \log (N) + b$$

$$N = 10^{\frac{(C_T - b)}{a}}$$

C_T	Threshold Cycle
a	Slope
N	copy number [copies/µl]
b	Intercept

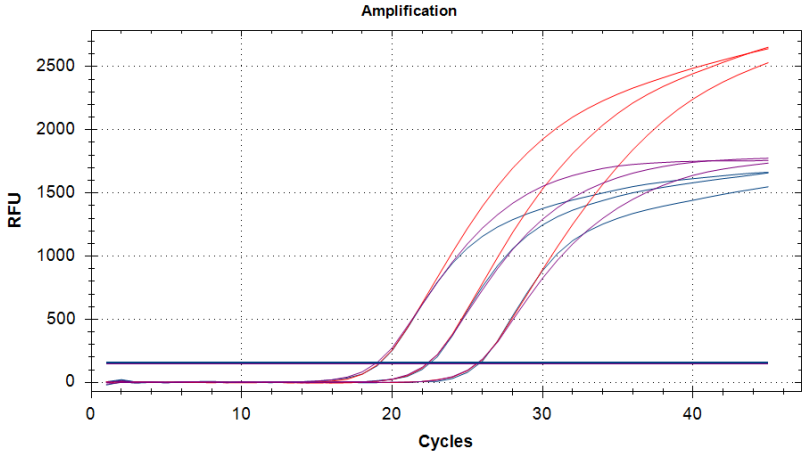

Figure 1: The amplification curves of the three standards for Lactobacillus species (FAM channel, blue), Gardnerella vaginalis (ROX channel, red) and Atopobium vaginae (Cy5 channel, purple) with 100,000 copies per µl (Standard 1), 10,000 copies per µl (Standard 2) and 1,000 copies per µl (Standard 3).

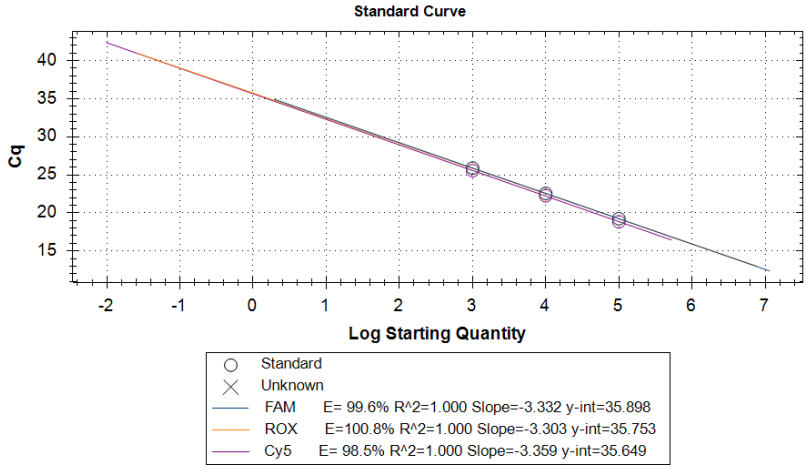

Figure 2: The C_T values of the three standards for Lactobacillus species (FAM channel), Gardnerella vaginalis (ROX channel) and Atopobium vaginae (Cy5 channel) plotted against the target copies per µl.

Interpretation of the Quantitative Analysis

The diagnosis of bacterial vaginosis is dependent on the proportions of the different bacteria in relation to each other. This is usually addressed by the Amsel criteria and the Nugent score, both microscopic methods. Up to now, there is no strict rule how qPCR matches those methods, but recent publications indicate Atopobium vaginae and Gardnerella vaginalis in comparison to Lactobacilli as useful to identify bacterial vaginosis [4].

To show potential outcomes of the diarellaBV-1 real time PCR, 4 samples are listed in Table 7. The corresponding amplification curves are presented in the following figures.

Table 7: Samples for the outcome of the quantitative analyses.

	Lactobacillus species copies/µl	**Gardnerella vaginalis** copies/µl	**Atopobium vaginae** copies/µl	Percentage Lactobacillus species
Sample A	1.4×10^6	-	-	100%
Sample B	1.025×10^6	1.003×10^5	-	90.8%
Sample C	9.719×10^3	1.090×10^3	5.236×10^5	1.7%
Sample D	1.66×10^3	8.331×10^4	-	2.3%

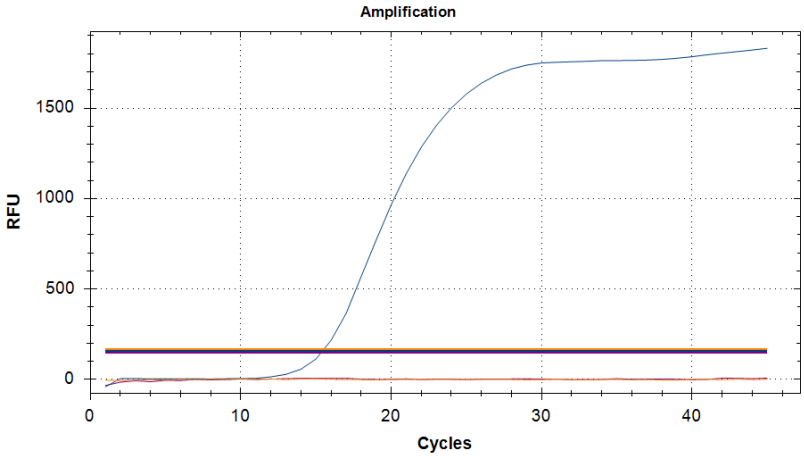

Figure 3: Sample A, only Lactobacillus species (blue) are present, the equilibrium of the vaginal microbiome is not affected by Atopobium vaginae or Gardnerella vaginalis.

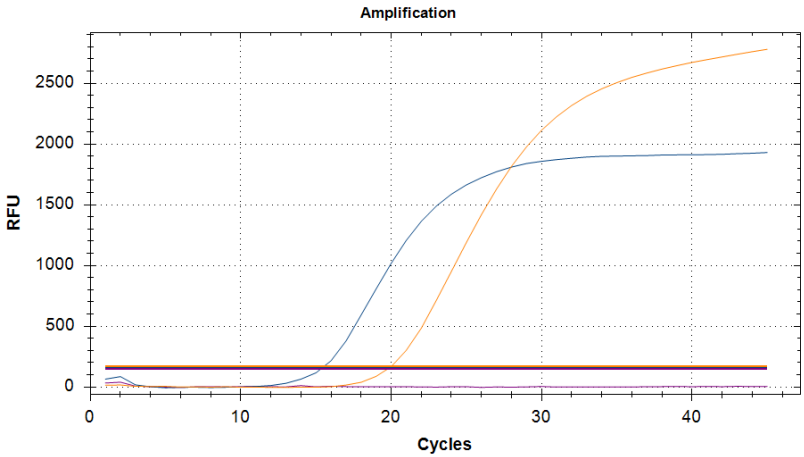

Figure 4: Sample B, high copy numbers for Lactobacillus species (blue) and Gardnerella vaginalis (orange). Lactobacillus species represent still 90.8% of the detected bacteria. The equilibrium of the vaginal microbiome seems to be unaffected or only slightly affected by the presence of Gardnerella vaginalis.

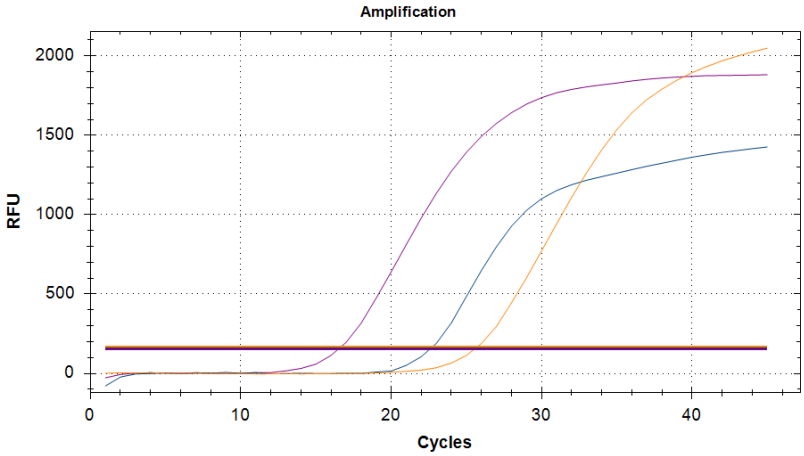

Figure 5: Sample C, very high copy numbers for Atopobium vaginae (purple). Medium copy numbers for Lactobacillus species (blue) and Gardnerella vaginalis (orange). Lactobacillus species represent 1.7% of the detected bacteria. The equilibrium of the vaginal microbiome seems to be affected, especially by Atopobium vaginae.

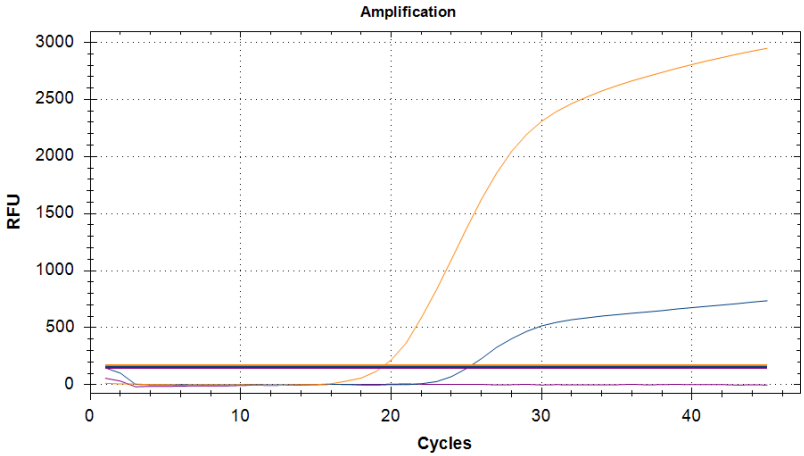

Figure 6: Sample D, high copy numbers for Gardnerella vaginalis (orange). Medium copy numbers for Lactobacillus species (blue). Lactobacillus species represent 2.3% of the detected bacteria. The equilibrium of the vaginal microbiome seems to be intermediate affected by Gardnerella vaginalis.

13 Assay Validation

Negative Control

The Negative Control must show no C_T in the FAM, Cy5, ROX and HEX channel.

Positive Control

The Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5 and ROX. The Positive Control must fall below a C_T of 30.

Internal Control

The following values for the amplification of the Internal Control are valid using gerbion nucleic acid extraction kit NukEx Mag RNA/DNA. The Control DNA (IPC) must show a positive (i.e. exponential) amplification curve.

The value of the IPC must be below a C_T of 34. If the IPC is above C_T 34 this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The value of the IPC in the water control must be below a C_T of 34.

If other nucleic acid extraction kits are used, the customer must define own cut offs. In this case the C_T value of the Control DNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

14 Limitations of the Method

- Strict compliance with the Instruction for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to use of this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the diarellaBV-1 real time PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM, Cy5, ROX channel of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of Lactobacillus specific amplification, the Cy5 channel for the A. vaginae specific amplification, the ROX channel for the G. vaginalis specific amplification and the HEX channel for the amplification of the Control DNA (IPC).
Incorrect configuration of the real time PCR	Check your work steps and compare with chapter 'Procedure'.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol described in chapter ´Instrument Settings´.
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter 'Transport, Storage and Stability'.

Weak or no signal of the Control DNA (IPC) and simultaneous absence of a signal in the specific FAM and/or Cy5 and/or ROX channel	
real time PCR conditions do not comply with the protocol	Check the real time PCR conditions (chapter ´Real time PCR´).
real time PCR inhibited	Make sure that you use an appropriate isolation method (see chapter 'Sample Preparation´) and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed.
sample material not sufficient	Make sure that enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter ´Sample Preparation´) and follow the manufacturer's instructions.
DNA loss during isolation process	In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter 'Transport, Storage and Stability'.
Detection of a fluorescence signal in the FAM channel of the Negative Control	
Contamination with Lactobacillus species during preparation of the PCR	Lactobacilli are part of the human skin microbiome and present in huge numbers. If the detected fluorescence signal shows a C_T value ≥ 35, it can be ignored. A C_T value ≥ 35 represents approximately 1 copy of Lactobacillus per µl of sample. This does not affect the interpretation of the result.
Detection of a fluorescence signal in the Cy5 and/or ROX channel of the Negative Control	
Contamination during preparation of the PCR	Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR.

16 Kit Performance

16.1 Analytical Sensitivity

For the FAM, ROX and Cy5 channel, the limit of detection (LoD) of diarellaBV-1 real time PCR Kit was determined using serial dilutions of synthetic DNA-fragments containing the specific gene target sequences. The determination of the LoD was done on a CFX Opus 96 Instrument (Bio-Rad).

The LoD of diarellaBV-1 real time PCR Kit is ≤ 2.5 genome copies per µl for the FAM, Cy5 and ROX channel.

16.2 Analytical Specificity

The specificity of the diarellaBV-1 real time PCR was evaluated with different ring trial samples of known status and different other relevant viruses and bacteria found in biological samples and basing on in silico analyses.
All ring trial samples and other eluates with known status were detected correctly. Results are shown in Table 8 and Table 9.

Table 8: Ring trial samples tested for the validation of the sensitivity and specificity of the diarellaBV-1 real time PCR Kit.

sample	Lactobacillus species	Atopobium vaginae	Gardnerella vaginalis
	FAM channel	Cy5 channel	ROX channel
QCMD 2020 Sexually Transmitted Infections I			
STI_I101S-01 Trichomonas vaginalis	negative	negative	negative
STI_I101S-02 Mycoplasma hominis	negative	negative	negative
STI_I101S-03 G. vaginalis + T. vaginalis	negative	negative	**positive**
STI_I101S-04 M. genitalium (drug resistant)	negative	negative	negative
STI_I101S-05 M. genitalium (wild type)	negative	negative	negative
STI_I101S-06 Negative	negative	negative	negative
STI_I101S-07 Gardnerella vaginalis	negative	negative	**positive**
STI_I101S-08 Trichomonas vaginalis	negative	negative	negative
STI_I101S-09 M. hominis + C. trachomatis	negative	negative	negative
STI_I101S-10 Trichomonas vaginalis	negative	negative	negative

Table 9: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaBV-1 real time PCR Kit.

Eluates with known status	**diarellaBV-1** Lactobacillus species **FAM channel**	**diarellaBV-1** Atopobium vaginae **Cy5 channel**	**diarellaBV-1** Gardnerella vaginalis **ROX channel**
Chlamydia pneumoniae	negative	negative	negative
Chlamydia trachomatis	negative	negative	negative
Cytomegalievirus	negative	negative	negative
Gardnerella vaginalis	negative	negative	**positive**
Herpes Simplex Virus Type 1	negative	negative	negative
Herpes Simplex Virus Type 2	negative	negative	negative
Mycoplasma genitalium	negative	negative	negative
Mycoplasma hominis	negative	negative	negative
Mycoplasma pneumoniae	negative	negative	negative
Neisseria gonorrhoeae	negative	negative	negative
Trichomonas vaginalis	negative	negative	negative
Ureaplasma parvum	negative	negative	negative
Ureaplasma urealyticum	negative	negative	negative
Varicella zoster virus Genotype 3	negative	negative	negative
Varicella zoster virus Genotype 5	negative	negative	negative

16.3 Linear Range

The linear range of the diarellaBV-1 real time PCR Kit was evaluated by analysing logarithmic dilution series of quantified synthetic DNAs of the target sequences.

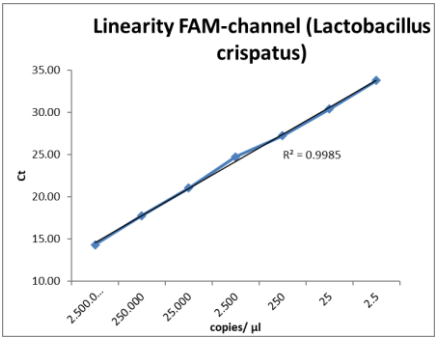

Figure 7: Determination of the linear range of the diarellaBV-1 real time PCR Kit for Lactobacillus crispatus in the FAM channel.

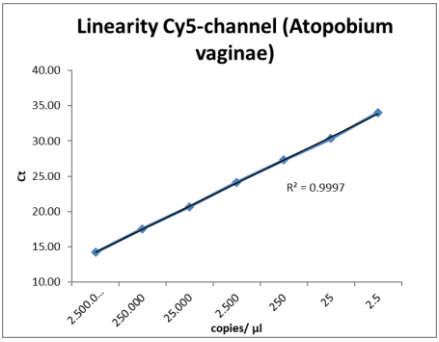

Figure 8: Determination of the linear range of the diarellaBV-1 real time PCR Kit for Atopobium vaginae in the Cy5 channel.

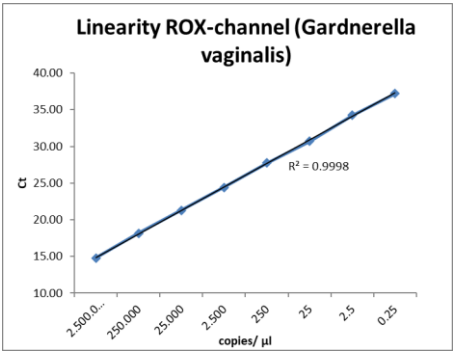

Figure 9: Determination of the linear range of the diarellaBV-1 real time PCR Kit for Gardnerella vaginalis in the ROX channel.

16.4 Precision

The precision of the diarellaBV-1 real time PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.
Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of Lactobacillus specific synthetic DNA, A. vaginae specific synthetic DNA and G. vaginalis specific synthetic DNA and additionally on the threshold cycle of the Control DNA (IPC). The results are shown in Table 10.

Table 10: Precision of the diarellaBV-1 real time PCR Kit

Lactobacillus crispatus (FAM)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.36	1.05
Inter-Assay Variability	2.5	0.41	1.22
Inter-Lot Variability	2.5	0.09	0.27
Atopobium vaginae (Cy5)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.37	1.07
Inter-Assay Variability	2.5	0.32	0.96
Inter-Lot Variability	2.5	0.44	1.28
Gardnerella vaginalis (ROX)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.43	1.25
Inter-Assay Variability	2.5	0.20	0.59
Inter-Lot Variability	2.5	0.03	0.10
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2500	0.09	0.33
Inter-Assay Variability	2500	0.20	0.70
Inter-Lot Variability	2500	0.07	0.24

16.5 Diagnostic Sensitivity

The diagnostic sensitivity of real time (RT-)PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the gerbion real time (RT-)PCR kits. gerbion real time (RT-)PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the real time (RT-)PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, gerbion guarantees the analytical sensitivities and specificities of the real time (RT-)PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. gerbion does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of gerbion real time (RT-)PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods. It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid	REF	Catalog number
PCR	Polymerase Chain Reaction	Σ	Content sufficient for <n> tests
REACTION MIX	Reaction Mix	-18°C	Upper limit of temperature
CONTROL +	Positive Control		Manufacturer
CONTROL −	Negative Control		Use by YYYY-MM-DD
CONTROL DNA IPC	Control DNA (IPC)	LOT	Batch code
STANDARD 1	Standard 1	IVD	*In vitro* diagnostic medical device
STANDARD 2	Standard 2	CE	European Conformity
STANDARD 3	Standard 3	UDI	Unique Device Identification
CONT	Content		Consult Instruction for Use

18 Literature

[1] Schwebke et al. (2014). Role of Gardnerella vaginalis in the Pathogenesis of Bacterial Vaginosis: A Conceptual Model. JID 2014:210 (1. August)

[2] Hickey et al. (2014). Gardnerella vaginalis Does Not Always Cause Bacterial Vaginosis. JID 2014:210 (15. November)

[3] Polatti (2012). Bacterial Vaginosis, Atopobium vaginae and Nifuratel. CCP 2012, 7, 36-40

[4] Loquet et al. (2021). Classification and Regression Trees for Bacterial Vaginosis Diagnosis in Pregnant Women Based on High-Throughput Quantitative PCR. JMD Vol.23 No.2 (February)