RUO

INSTRUCTION FOR USE

Women Health Panel PCR Kit

For Research Use Only

100

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CONTENTS

1. INTENDED USE

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease. Furthermore, the test kit is not intended to diagnose infectious animal diseases.

The ***MarinaBiolab Women Health Panel PCR Kit*** is a multiplexed qualitative Real-Time Polymerase Chain Reaction (qPCR) test intended for the simultaneous detection and identification of multiple pathogenic nucleic acids in research samples. The ***MarinaBiolab Women Health Panel PCR Kit*** allows to achieve qPCR result in less than 1 hour. The test is performed to detect gene sequences of the following organisms.

Targets	
Haemophilus ducreyi	*Prevotella bivia*
Bacterial Vaginosis Associated Bacteria 2	*Staphylococcus aureus*
Fannyhessea vaginae	*Mobiluncus curtisii*
Escherichia coli	*Mobiluncus mulieris*
Streptococcus agalactiae	*Gardnerella vaginalis*
Lactobacillus gasseri	*Enterococcus faecalis*
Lactobacillus iners	*Trichomonas vaginalis*
Lactobacillus crispatus	*Candida albicans*
Lactobacillus jensenii	*Candida glabrata*
Megasphaera phylotypes 1	*Candida parapsilosis*
Megasphaera phylotypes 2	*Candida tropicalis*
Controls	
Human RNase P (IC)	
Bacillus atrophaeus (EC)	

2. PRINCIPLE of the PROCEDURE

DNA target regions are amplified via real-time PCR instruments using the primer and probe sets in the kit. In the process, the probe anneals a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instruments. Probes labeled with different fluorophores are used to detect specific amplicons originating from targets and Internal Control.

PCR instruments measure these signals at the end of each amplification cycle in real time and interpret the data to provide a qualitative result for each of the above targets. A positive result for the detection of target DNA is indicated by the presence of a real-time PCR growth curve and an associated Cq (Quantification Cycle) value.

3. KIT COMPONENTS

The ***MarinaBiolab Women Health Panel PCR Kit*** consists of four main components:

1. qPCR Enzyme and Buffer Mix (qPCR Master Mix)
2. Forward, Reverse and Probe Oligo Mix (WHP Oligo Mix 1-8)
3. Mix of non-infectious DNA from artificial sample including targets in the table below (PC-WHP)
4. DNase/RNase-Free Water (NTC)

The kit components are provided in Table 1-2.

Table 1. Kit components.

Component	Description	Quantity x Volume
		100 rxn **MBLWMH006**
qPCR Master Mix	Ready-to-use mix for qPCR	4 x 1000 µL
WHP Oligo Mix 1-8	Primers and probes complementary to specific regions of the targets in the table above	8 x 250 µL
PC-WHP	Mix of non-infectious DNA from artificial sample including targets in the table above	2 x 400 µL
NTC	DNase/RNase-Free Water	2 x 400 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
WHP Oligo Mix 1	*Haemophilus ducreyi*	**FAM/Green**
	Bacterial Vaginosis Associated Bacteria 2	**HEX/VIC/JOE/Yellow**
	Fannyhessea vaginae	**ROX/Texas Red/Orange**
	Human RNase P (IC)	**CY5/Red**
WHP Oligo Mix 2	*Candida albicans*	**FAM/Green**
	Candida glabrata	**HEX/VIC/JOE/Yellow**
	Candida parapsilosis	**ROX/Texas Red/Orange**
	-	**CY5/Red**
WHP Oligo Mix 3	*Escherichia coli*	**FAM/Green**
	Streptococcus agalactiae	**HEX/VIC/JOE/Yellow**
	Prevotella bivia	**ROX/Texas Red/Orange**
	-	**CY5/Red**

WHP Oligo Mix 4	*Lactobacillus gasseri*	**FAM/Green**
	Lactobacillus iners	**HEX/VIC/JOE/Yellow**
	Lactobacillus crispatus	**ROX/Texas Red/Orange**
	-	**CY5/Red**
WHP Oligo Mix 5	-	**FAM/Green**
	Megasphaera phylotypes 1	**HEX/VIC/JOE/Yellow**
	Megasphaera phylotypes 2	**ROX/Texas Red/Orange**
	-	**CY5/Red**
WHP Oligo Mix 6	*Staphylococcus aureus*	**FAM/Green**
	Mobiluncus curtisii	**HEX/VIC/JOE/Yellow**
	Mobiluncus mulieris	**ROX/Texas Red/Orange**
	-	**CY5/Red**
WHP Oligo Mix 7	*Gardnerella vaginalis*	**FAM/Green**
	Enterococcus faecalis	**HEX/VIC/JOE/Yellow**
	Lactobacillus jensenii	**ROX/Texas Red/Orange**
	-	**CY5/Red**
WHP Oligo Mix 8	*Candida tropicalis*	**FAM/Green**
	Trichomonas vaginalis	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	Bacillus atrophaeus (EC)	**CY5/Red**

The oligonucleotide set targeting the human *RNase P* mRNA (Internal Control: IC) and *Bacillus atrophaeus* (External Control: EC) are used to monitor sampling, nucleic acid extraction, and inhibition of qPCR. The kit also contains negative and positive control templates for evaluating the contamination and the qPCR reagent stability, respectively.

4. EQUIPMENT and MATERIALS REQUIRED but NOT PROVIDED

- 2-8°C Refrigerator
- ≤ -20°C Freezer
- ≤ -70°C Freezer (Optional)
- Vortex mixer
- Benchtop centrifuge with rotor for 1.5 mL tubes
- Benchtop mini centrifuge with rotor for PCR strips
- Benchtop plate centrifuge
- Biological Safety Cabinet (BSC)
- PCR cabinet for PCR Setup
- Adjustable Micropipettes: 1-10, 10-100, 100-1000 µL
- Sterile DNase/RNase free micropipettes tips - Compatible with the micropipettes
- Cold tube rack for microfuge tubes (1.5/2 mL) and for PCR tubes (0.1/0.2 mL)
- Disposable, powder-free, nitrile gloves
- Disposable (preferably) laboratory coat
- Surface decontaminants - Freshly diluted 10% bleach solution (0.5% NaClO)
- Applied Biosystems QuantStudio 5, 7, and 12K with Design & Analysis software and consumables
- Bio-Rad CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/CFX384 Touch™/CFX Opus 384™ with Maestro software v1.1 and consumables
- Qiagen Rotor-Gene Q 5plex Platform with Rotor-Gene Q series software v2.1.0.9 and consumables
- Roche LightCycler 480 with software and consumables

5. WARNING and PRECAUTIONS

- The ***MarinaBiolab Women Health Panel PCR Kit*** is designed for research use only and should be used by professionally trained, qualified staff only. All work should be performed using Good Laboratory Practices.
- Biological material used for extraction of nucleic acid should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).
- Biological material should be inactivated before disposal (e.g., in an autoclave). Disposables should be autoclaved or incinerated after use.
- Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g., in an autoclave).
- Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state, and local regulations.
- Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one-way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.
- The kit should be stored away from nucleic acid sources and qPCR amplicons.
- Always check the expiration date on the kit. Do not use expired or incorrectly stored kit.
- The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
- Kit components should be mixed by gently shaking before use.
- A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. To prevent amplicon contamination:
    - It shall be ensured that separate work areas with their own apparatus are available.
    - Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
    - Discard used tubes/plates in a biohazard container immediately after the run has completed.
    - Avoid excessive handling of tubes/plates after test runs.
    - Change gloves after handling a used tubes/plate.

6. HANDLING, STORAGE, and STABILITY

- The ***MarinaBiolab Women Health Panel PCR Kit*** is shipped on dry ice. If any component except qPCR Master Mix of the kit is not frozen on arrival, or if the outer packaging has been compromised during shipment, please contact **MarinaBiolab** or the local distributors as soon as possible.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated freezing and thawing of the kit components may result in lower detection quality. The kit can undergo up to 15 freeze/thaw cycles without affecting performance.
- When stored under the specified storage conditions, the kit is stable until the stated expiration date printed on the package. The expiration date of the kit is 12 months from date of manufacture.
- All components must be thawed at ambient temperature for a minimum of 30 minutes before use.
- It is recommended that all components should be kept on ice when setting up the assay mixes.
- The primer and probe mixes contain fluorophore labeled probes and should be protected from direct sunlight or long-term ambient light.
- Do not use expired or incorrectly stored components.

7. TEST PROCEDURE

7.1. Sample Preparation and Nucleic Acid Extraction

The sample material for the isolation of nucleic acid must be sent in appropriate cell collection systems. The performance of the kit strongly depends on the amount and quality of the extracted nucleic acid. It must be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

If the established standard method of the lab is used for nucleic acid isolation, it must be validated by the user.

For frozen samples or frozen extracted nucleic acid, only thaw the number of specimen extracts that will be tested in a single day.

Do not freeze/thaw extracted nucleic acid more than once before testing as each freeze/thaw cycle can decrease the nucleic acid quality. For optimal results, use it directly.

7.2. PCR Reaction Preparation and Processing

- Completely thaw the components at room temperature for a minimum of 30 minutes before each use.
- Place all components on ice once thawed during the whole test procedure.
- Determine the number of reactions and create the PCR plate plan.
- Include the following reactions to the plan:
    - Reactions for each test sample and extraction negative control.
    - PCR control reactions:
        - Positive Control (included in the kit)
        - Negative (No Template) Control (NTC) (included in the kit)
        - No Template Addition Control (NRC)
- Vortex and spin down briefly the components before each use.
- Combine the following components for the number of reactions required plus 10% overage to compensate for pipetting errors:

Table 3. Reaction set-up.

Reaction Mix Component	1X Reaction (µL) per well
qPCR Master Mix	5 µL
WHP Oligo Mix 1-8	2.5 µL
Template Nucleic Acid	2.5 µL
Total Reaction Volume	10 µL

- Add 5 µL of qPCR Master Mix and 2.5 µL of WHP Oligo Mix 1-8 into PCR tubes.
- Add 2.5 µL of the isolated sample into the individual tubes.
- The final reaction mix volume is 10 µL.
- Close the tubes, centrifuge briefly, insert tubes into the real-time PCR instrument and amplify according to the following PCR profile.

Table 4. Amplification profile.

Step	Number of Cycles	Temperature	Time	Data Collection
Initial Denaturation	1	95 °C	10 sec	**FAM/Green**, **HEX/VIC/JOE/Yellow**, **ROX/Texas Red/Orange**, **CY5/Red**
Denaturation	40	95 °C	5 sec	
Annealing/Extension		55 °C	15 sec	

8. INTERPRETATION OF RESULTS

MarinaBiolab Women Health Panel PCR Kit provides a qualitative result for the presence (Detected) or absence (Not Detected) of the target genes.

8.1. Calculation of Cq Values and Instrument-Specific Requirements

Perform the following instrument settings before evaluating the results.

Table 5. Instrument-specific requirements before evaluating the results.

Instrument	Threshold Level	Other Settings
CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/ CFX384 Touch™/CFX Opus 384™ (Bio-Rad)	500 RFU	-
Rotor-Gene Q 5plex Platform (QIAGEN)	0.02 RFU	Dynamic Tube: Active Slope Correct: Active Outlier Removal: 0
QuantStudio™ 5, 7 and 12K (Applied Biosystems™)	Auto	-
Roche LightCycler 480 (Roche)	Auto	-

The shape of the amplification curves should be examined. If a Cq value is assigned to a sample by the instruments' software and the curve is sigmoidal, the Cq value can be used in the final evaluation. *Non-sigmoidal curves should be recorded as negative.*

The result is recorded as positive if Cq≤38 or as established by your lab.

8.2. Overall Validity of Detection

Table 6. Expected performance of controls.

Control Type	Used to Monitor	Signal	
		Target Channel	Internal/External Control Channel
Negative Control	Cross-contamination during extraction and reaction setup	-	-
No template addition	Reagent and/or environmental contamination	-	-
Positive Control	qPCR reaction setup and reagent integrity	+	+
Internal/External Control	To monitor the integrity of nucleic acid extraction and qPCR from each specimen	Not applicable	+

Before analyzing samples results, we recommend verifying if the real-time PCR test is valid. Thus, for each run, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

Table 7. Run validity/positive and negative control pass criteria.

Positive Control		Negative Control		Results	Recommendation
Target Channel	**Internal/External Control Channel**	**Target Channel**	**Internal/External Control Channel**		
+	+	-	-	VALID	Continue to result interpretation of samples.
Any of them is Negative		Not considered		INVALID	Contact the manufacturer, renew the reagents, and repeat the reaction.
Not considered		Any of them is Positive		INVALID	Repeat analysis, paying attention to "Warnings and Precautions" in IFU.

If any control does not perform as described above, the run is considered invalid, and the test is repeated. If the problem persists, contact the manufacturer.

If all the controls are valid, proceed to the interpretation of the results.

8.3. Interpretation of Unknown Specimen Results

The data produced by the instruments can manually be evaluated and reported using their software.

Table 8. Interpretation of unknown specimen results for DNA pathogens.

DNA Pathogens	Internal Control (*RNase P*)	External Control (*Bacillus atrophaeus*)	Results	Interpretation
Positive (+) (Cq<38)	Positive (+) (Cq<38)	Positive (+) (Cq<38)	Positive for Target	Target DNA is detected
Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Positive for Target	Target DNA is detected
Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Positive (+) (Cq<38)	Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Positive (+) (Cq<38)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.

9. ASSAY LIMITATIONS

- The ***MarinaBiolab Women Health Panel PCR Kit*** is intended for use by professionally trained, qualified staff only.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Spontaneous mutations within the target sequences may result in failure to detect the target sequence. While this risk is mitigated in the test's design, if failure to detect the target is expected it is recommended to test the specimen with a different test that detects different target sequences from the target's genome.
- There is a risk of false positive results due to cross-contamination by target viruses and/or bacteria, their nucleic acids or amplified product, or from non-specific signals in the assay. Attention should be given to the handling of consumables under the Warnings and Precautions section to help minimize this risk.
- This assay is a qualitative test and does not provide a quantitative assessment of the concentration of the detected organism.
- All instruments (e.g., pipettes, real-time cyclers) must be calibrated according to the manufacturer's instructions.

10. PERFORMANCE CHARACTERISTICS

10.1. Analytical Sensitivity (Limit of Detection, LoD)

The LoD was defined as the concentration at which the test produces a positive result >95% of the time. Serial dilutions of the strains were tested and the initial tentative LoD confirmed with twenty (20) replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further twenty (20) replicates were performed at the next lower concentration until ≤95% was achieved. For nucleic acid extraction, simulated research matrix was spiked with strains and loaded onto the Automatic Nucleic Acids Extraction Instrument. The tests were carried out using the CFX96 Touch™ (Bio-Rad) Real-Time PCR system. The confirmed LoDs for the strains tested and the corresponding LoDs for the ***MarinaBiolab Women Health Panel PCR Kit*** reportable targets are shown in Table 9 below.

Table 9. Summary of LoD study results.

Analyte	Isolate ID/Source	LoD Concentration (copies/mL)	Detected/Total
Haemophilus ducreyi	Zeptometrix 0801736DNA	2.5E+01 copies/mL	**20/20** 100%
Bacterial Vaginosis Associated Bacteria 2	ATCC TSD-275	6.7+01 copies/mL	**20/20** 100%
Fannyhessea vaginae	ATCC BAA-55	7.8+01 copies/mL	**20/20** 100%
Candida albicans	ATCC 10231	3.4E+02 copies/mL	**20/20** 100%
Candida glabrata	ATCC 90030	4.4E+01 copies/mL	**20/20** 100%
Candida parapsilosis	ATCC 22019	5.8E+01 copies/mL	**20/20** 100%
Escherichia coli	ATCC 25922	3.5E+01 copies/mL	**20/20** 100%
Streptococcus agalactiae	ATCC 12386	6.7E+01 copies/mL	**19/20** 95%
Prevotella bivia	Zeptometrix 0801756	8.9+01 copies/mL	**20/20** 100%
Lactobacillus gasseri	Zeptometrix 0804327	1.1+02 copies/mL	**20/20** 100%
Lactobacillus iners	Zeptometrix 0804261	7.7+01 copies/mL	**20/20** 100%
Lactobacillus crispatus	Zeptometrix 0804143	9.5+01 copies/mL	**20/20** 100%
Megasphaera phylotypes 1	In-house	2.1+02 copies/mL	**20/20** 100%

Megasphaera phylotypes 2	In-house	1.9+02 copies/mL	**20/20** 100%
Staphylococcus aureus	ATCC 12600	8.6E+01 copies/mL	**20/20** 100%
Mobiluncus curtisii	Zeptometrix 0804141	8.9+01 copies/mL	**20/20** 100%
Mobiluncus mulieris	Zeptometrix 0804116	9.1+01 copies/mL	**20/20** 100%
Gardnerella vaginalis	ATCC 49145	1.5E+01 copies/mL	**20/20** 100%
Enterococcus faecalis	Zeptometrix 0804216	3.6E+02 copies/mL	**20/20** 100%
Lactobacillus jensenii	Zeptometrix 0804260	8.5+01 copies/mL	**20/20** 100%
Candida tropicalis	ATCC 750	5.7E+01 copies/mL	**20/20** 100%
Trichomonas vaginalis	ATCC 30001	2.5E+01 copies/mL	**20/20** 100%

10.2. Device Equivalence Study

Device equivalence study was carried out to observe the differences between the results to be obtained using the kit in different instruments. For this purpose, the same LoD determination study was performed again with the Bio-Rad CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/CFX384 Touch™/CFX Opus 384™, Applied Biosystems QuantStudio 5, 7, and 12K, Qiagen Rotor-Gene Q 5plex Platform and Roche LightCycler 480. Similar test results were obtained with the 1x LoD concentration level of the targets in the "device equivalence study" performed with the other instruments.

10.3. Analytical Reactivity (Inclusivity)

10.3.1. In-Slico Analytical Reactivity

BLAST search of the oligonucleotides was performed on the *Haemophilus ducreyi, Bacterial Vaginosis Associated Bacteria 2, Fannyhessea vaginae, Candida albicans, Candida glabrata, Candida parapsilosis, Escherichia coli, Streptococcus agalactiae, Prevotella bivia, Lactobacillus gasseri, Lactobacillus iners, Lactobacillus crispatus, Megasphaera phylotypes 1, Megasphaera phylotypes 2, Staphylococcus aureus, Mobiluncus curtisii, Mobiluncus mulieris, Gardnerella vaginalis, Enterococcus faecalis, Lactobacillus jensenii, Candida tropicalis,* and *Trichomonas vaginalis* genome sequences available in the NCBI database, using the Primer-BLAST tool of NCBI.

The aggregated result of all in-silico analyzes performed in NCBI database is provided in Table below. The melting temperatures (Tm) of the oligonucleotide sequences with 1-base mismatch, are still higher than the annealing temperature specified in the PCR cycle parameters of the kit. Hence, the single mismatches in the sequences are not expected to affect the inclusivity of the test.

Table 10. In-silico analysis results performed in the NCBI database.

Target	Primer	Total number of target sequences	Ratio of the sequences without mismatch	Ratio of the sequences with 1 base mismatch	Ratio of the sequences with 2 base mismatches	Ratio of the sequences with 3 base mismatches
Haemophilus ducreyi	Sense Primer	40	100.00%	0.00%	0.00%	0.00%
Haemophilus ducreyi	Antisense Primer	40	100.00%	0.00%	0.00%	0.00%
Haemophilus ducreyi	Hydrolysis Probe	40	100.00%	0.00%	0.00%	0.00%
Bacterial Vaginosis Associated Bacteria 2	Sense Primer	24	100.00%	0.00%	0.00%	0.00%
Bacterial Vaginosis Associated Bacteria 2	Antisense Primer	24	100.00%	0.00%	0.00%	0.00%
Bacterial Vaginosis Associated Bacteria 2	Hydrolysis Probe	24	100.00%	0.00%	0.00%	0.00%
Fannyhessea vaginae	Sense Primer	44	100.00%	0.00%	0.00%	0.00%
Fannyhessea vaginae	Antisense Primer	44	100.00%	0.00%	0.00%	0.00%
Fannyhessea vaginae	Hydrolysis Probe	44	100.00%	0.00%	0.00%	0.00%
Candida albicans	Sense Primer	3.629	99.69%	0.31%	0.00%	0.00%
Candida albicans	Antisense Primer	3.728	98.85%	2.25%	0.00%	0.00%
Candida albicans	Hydrolysis Probe	3.728	98.52%	2.48%	0.00%	0.00%
Candida glabrata	Sense Primer	763	100%	0.00%	0.00%	0.00%
Candida glabrata	Antisense Primer	1.111	99.20%	0.80%	0.00%	0.00%
Candida glabrata	Hydrolysis Probe	1.111	99.64%	0.36%	0.00%	0.00%
Candida parapsilosis	Sense Primer	2.559	99.74%	0.26%	0.00%	0.00%
Candida parapsilosis	Antisense Primer	2.463	100%	0.00%	0.00%	0.00%
Candida parapsilosis	Hydrolysis Probe	2.463	100%	0.00%	0.00%	0.00%
Escherichia coli	Sense Primer	5.547	99.25%	0.75%	0.00%	0.00%
Escherichia coli	Antisense Primer	5.579	99.65%	0.35%	0.00%	0.00%
Escherichia coli	Hydrolysis Probe	5.579	99.78%	0.22%	0.00%	0.00%
Streptococcus agalactiae	Sense Primer	226	99.95%	0.05%	0.00%	0.00%
Streptococcus agalactiae	Antisense Primer	236	100.00%	0.00%	0.00%	0.00%
Streptococcus agalactiae	Hydrolysis Probe	236	100.00%	0.00%	0.00%	0.00%
Prevotella bivia	Sense Primer	56	99.80%	0.20%	0.00%	0.00%
Prevotella bivia	Antisense Primer	56	99.80%	0.20%	0.00%	0.00%
Prevotella bivia	Hydrolysis Probe	52	99.60%	0.40%	0.00%	0.00%

Lactobacillus gasseri	Sense Primer	987	98.24%	1.76%	0.00%	0.00%
Lactobacillus gasseri	Antisense Primer	987	98.24%	1.76%	0.00%	0.00%
Lactobacillus gasseri	Hydrolysis Probe	956	98.10%	1.90%	0.00%	0.00%
Lactobacillus iners	Sense Primer	1458	97.68%	2.00%	0.32%	0.00%
Lactobacillus iners	Antisense Primer	1458	97.68%	2.00%	0.32%	0.00%
Lactobacillus iners	Hydrolysis Probe	1402	97.70%	2.20%	0.10%	0.00%
Lactobacillus crispatus	Sense Primer	825	98.21%	1.79%	0.00%	0.00%
Lactobacillus crispatus	Antisense Primer	825	98.21%	1.79%	0.00%	0.00%
Lactobacillus crispatus	Hydrolysis Probe	828	98.11%	1.89%	0.00%	0.00%
Megasphaera phylotypes 1	Sense Primer	54	99.80%	0.20%	0.00%	0.00%
Megasphaera phylotypes 1	Antisense Primer	54	99.80%	0.20%	0.00%	0.00%
Megasphaera phylotypes 1	Hydrolysis Probe	53	99.60%	0.40%	0.00%	0.00%
Megasphaera phylotypes 2	Sense Primer	20	100.00%	0.00%	0.00%	0.00%
Megasphaera phylotypes 2	Antisense Primer	20	100.00%	0.00%	0.00%	0.00%
Megasphaera phylotypes 2	Hydrolysis Probe	20	100.00%	0.00%	0.00%	0.00%
Staphylococcus aureus	Sense Primer	657	99.80%	0.20%	0.00%	0.00%
Staphylococcus aureus	Antisense Primer	657	99.80%	0.20%	0.00%	0.00%
Staphylococcus aureus	Hydrolysis Probe	655	99.70%	0.30%	0.00%	0.00%
Mobiluncus curtisii	Sense Primer	34	100.00%	0.00%	0.00%	0.00%
Mobiluncus curtisii	Antisense Primer	34	100.00%	0.00%	0.00%	0.00%
Mobiluncus curtisii	Hydrolysis Probe	34	100.00%	0.00%	0.00%	0.00%
Mobiluncus mulieris	Sense Primer	20	100.00%	0.00%	0.00%	0.00%
Mobiluncus mulieris	Antisense Primer	20	100.00%	0.00%	0.00%	0.00%
Mobiluncus mulieris	Hydrolysis Probe	20	100.00%	0.00%	0.00%	0.00%
Gardnerella vaginalis	Sense Primer	52	100.00%	0.00%	0.00%	0.00%
Gardnerella vaginalis	Antisense Primer	52	100.00%	0.00%	0.00%	0.00%
Gardnerella vaginalis	Hydrolysis Probe	50	100.00%	0.00%	0.00%	0.00%
Enterococcus faecalis	Sense Primer	575	100.00%	0.00%	0.00%	0.00%
Enterococcus faecalis	Antisense Primer	578	100.00%	0.00%	0.00%	0.00%
Enterococcus faecalis	Hydrolysis Probe	578	99.89%	0.11%	0.00%	0.00%
Lactobacillus jensenii	Sense Primer	687	98.23%	2.67%	0.00%	0.00%
Lactobacillus jensenii	Antisense Primer	687	98.23%	2.67%	0.00%	0.00%

Lactobacillus jensenii	Hydrolysis Probe	660	98.07%	2.93%	0.00%	0.00%
Candida tropicalis	Sense Primer	1.164	98.40%	2.60%	0.00%	0.00%
Candida tropicalis	Antisense Primer	1.906	97.83%	2.17%	0.00%	0.00%
Candida tropicalis	Hydrolysis Probe	1.906	97.12%	2.88%	0.00%	0.00%
Trichomonas vaginalis	Sense Primer	63	99.79%	0.21%	0.00%	0.00%
Trichomonas vaginalis	Antisense Primer	63	99.79%	0.21%	0.00%	0.00%
Trichomonas vaginalis	Hydrolysis Probe	60	99.75%	0.25%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab Women Health Panel PCR Kit*** was demonstrated with a comprehensive panel representing temporal, evolutionary, and geographic diversity for each of the target organisms.

Each sample was tested with the ***MarinaBiolab Women Health Panel PCR Kit*** in triplicate at an initial concentration 3-fold higher than the LoD determined for each analyte. In cases where the expected targets were not detected in one or more replicates, concentrations at a 3-fold higher level were evaluated.

The individual strains and concentrations at which positive test results were obtained for all three (3) replicates are presented by target organism in Table 11 below.

Table 11. Results of the wet inclusivity test.

Variant/Type/Subtype/Lineage/Genotype/Species	Isolate ID/Source	xLoD Detected
Haemophilus ducreyi	Zeptometrix 0801736DNA	1x
Bacterial Vaginosis Associated Bacteria 2	ATCC TSD-275	1x
Fannyhessea vaginae	ATCC BAA-55	1x
Candida albicans	ATCC 10231	1x
Candida glabrata	ATCC 90030	1x
Candida parapsilosis	ATCC 22019	1x
Escherichia coli	ATCC 25922	1x
Streptococcus agalactiae	ATCC 12386	1x
Prevotella bivia	Zeptometrix 0801756	1x
Lactobacillus gasseri	Zeptometrix 0804327	1x
Lactobacillus iners	Zeptometrix 0804261	1x
Lactobacillus crispatus	Zeptometrix 0804143	1x
Megasphaera phylotypes 1	In-house	1x
Megasphaera phylotypes 2	In-house	1x

Staphylococcus aureus	ATCC 12600	1x
Mobiluncus curtisii	Zeptometrix 0804141	1x
Mobiluncus mulieris	Zeptometrix 0804116	1x
Gardnerella vaginalis	ATCC 49145	1x
Enterococcus faecalis	Zeptometrix 0804216	1x
Lactobacillus jensenii	Zeptometrix 0804260	1x
Candida tropicalis	ATCC 750	1x
Trichomonas vaginalis	ATCC 30001	1x

10.4. Analytical Specificity (Exclusivity)

10.4.1. In-Slico Analytical Specificity

Primers and probes intended for a target sequence may also attach to similar sequences if they closely match or differ by only a few base pairs from the non-targeted sequence. To ensure specificity to the target amplicon sequence, it's essential to screen the primers and probe against the reference database transcript or genome database for the intended templates, as well as any databases containing potential contaminating templates.

Table 12. The results of On-Panel and Off-Panel organisms tested for cross-reactivity.

On-Panel/Off-Panel	Name of the organism	Cross Reactivity*		
		Forward	Probe	Reverse
On-Panel	*Haemophilus ducreyi*	None	None	None
On-Panel	*Bacterial Vaginosis Associated Bacteria 2*	None	None	None
On-Panel	*Fannyhessea vaginae*	None	None	None
On-Panel	*Candida albicans*	None	None	None
On-Panel	*Candida glabrata*	None	None	None
On-Panel	*Candida parapsilosis*	None	None	None
On-Panel	*Escherichia coli*	None	None	None
On-Panel	*Streptococcus agalactiae*	None	None	None
On-Panel	*Prevotella bivia*	None	None	None
On-Panel	*Lactobacillus gasseri*	None	None	None
On-Panel	*Lactobacillus iners*	None	None	None
On-Panel	*Lactobacillus crispatus*	None	None	None
On-Panel	*Megasphaera phylotypes 1*	None	None	None
On-Panel	*Megasphaera phylotypes 2*	None	None	None

On-Panel	*Staphylococcus aureus*	None	None	None
On-Panel	*Mobiluncus curtisii*	None	None	None
On-Panel	*Mobiluncus mulieris*	None	None	None
On-Panel	*Gardnerella vaginalis*	None	None	None
On-Panel	*Enterococcus faecalis*	None	None	None
On-Panel	*Lactobacillus jensenii*	None	None	None
On-Panel	*Candida tropicalis*	None	None	None
On-Panel	*Trichomonas vaginalis*	None	None	None
Off-Panel	*Acinetobacter calcoaceticus*	None	None	None
Off-Panel	*Acinetobacter baumannii*	None	None	None
Off-Panel	*Serratia marcescens*	None	None	None
Off-Panel	*Klebsiella aerogenes*	None	None	None
Off-Panel	*Klebsiella oxytoca*	None	None	None
Off-Panel	*Staphylococcus saprophyticus*	None	None	None
Off-Panel	*Klebsiella pneumoniae*	None	None	None
Off-Panel	*Proteus mirabilis*	None	None	None
Off-Panel	*Proteus vulgaris*	None	None	None
Off-Panel	*Morganella morganii*	None	None	None
Off-Panel	*Citrobacter freundii*	None	None	None
Off-Panel	*Aerococcus urinae*	None	None	None
Off-Panel	*Bacteroides fragilis*	None	None	None
Off-Panel	*Neisseria meningitidis*	None	None	None
Off-Panel	Human papillomavirus 16	None	None	None
Off-Panel	Human papillomavirus 18	None	None	None
Off-Panel	Human papillomavirus type 52	None	None	None
Off-Panel	Human papillomavirus 6	None	None	None
Off-Panel	Human papillomavirus 11	None	None	None
Off-Panel	Human papillomavirus type 58	None	None	None
Off-Panel	Human papillomavirus type 33	None	None	None

* Homology should be <80% between the cross-reactivity microorganisms and the test primers/ probe(s).

** In silico sequence analysis indicates the potential for cross-reactivity of Bordetella pertussis with certain strains of Bordetella bronchiseptica.

10.4.2. Wet-Test Analytical Specificity

The potential for non-specific amplification by assays for detection of analytes was evaluated by testing high concentrations of organisms or nucleic acids with the ***MarinaBiolab Women Health Panel PCR Kit***. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity, and off-panel organisms were tested to evaluate panel specificity. Off-panel organisms included normal flora and pathogens that may be present in specimens as well as near-neighbors or species genetically related to the organisms detected by the ***MarinaBiolab Women Health Panel PCR Kit***. The concentration of organism tested (in triplicate) was at least 1.0E+06 CFU/mL for bacteria, fungi and parasite, and at least 1.0E+05 unit/mL for viruses. For the few organisms of interest that were not available for laboratory testing, results of in silico analysis of the organism's whole genome sequences are indicated. The on-panel and off-panel organisms tested are shown in Table 13 and Table 14.

Table 13. On-Panel organisms tested for evaluation of ***MarinaBiolab Women Health Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Haemophilus ducreyi	Zeptometrix 0801736DNA	None
Bacterial Vaginosis Associated Bacteria 2	ATCC TSD-275	None
Fannyhessea vaginae	ATCC BAA-55	None
Candida albicans	ATCC 10231	None
Candida glabrata	ATCC 90030	None
Candida parapsilosis	ATCC 22019	None
Escherichia coli	ATCC 25922	None
Streptococcus agalactiae	ATCC 12386	None
Prevotella bivia	Zeptometrix 0801756	None
Lactobacillus gasseri	Zeptometrix 0804327	None
Lactobacillus iners	Zeptometrix 0804261	None
Lactobacillus crispatus	Zeptometrix 0804143	None
Megasphaera phylotypes 1	In-house	None
Megasphaera phylotypes 2	In-house	None
Staphylococcus aureus	ATCC 12600	None
Mobiluncus curtisii	Zeptometrix 0804141	None
Mobiluncus mulieris	Zeptometrix 0804116	None
Gardnerella vaginalis	ATCC 49145	None
Enterococcus faecalis	Zeptometrix 0804216	None
Lactobacillus jensenii	Zeptometrix 0804260	None
Candida tropicalis	ATCC 750	None
Trichomonas vaginalis	ATCC 30001	None

Table 14. Off-Panel organisms were tested for evaluation of ***MarinaBiolab Women Health Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Acinetobacter calcoaceticus	ATCC 23055	None
Acinetobacter baumannii	ATCC 19606	None
Serratia marcescens	ATCC 29021	None
Klebsiella aerogenes	ATCC 13048	None
Klebsiella oxytoca	ATCC 700324	None
Staphylococcus saprophyticus	Zeptometrix 0804014	None
Klebsiella pneumoniae	NCTC 13465	None
Proteus mirabilis	Zeptometrix 0801544	None
Proteus vulgaris	ATCC 6380	None
Morganella morganii	Zeptometrix 0804010	None
Citrobacter freundii	Zeptometrix 0801563	None
Aerococcus urinae	ATCC 51268	None
Bacteroides fragilis	ATCC 25285	None
Human papillomavirus 16	NIBSC-UK-EN63QG	None
Human papillomavirus 18	NIBSC-UK-EN63QG	None
Human papillomavirus type 52	NIBSC-UK-EN63QG	None
Human papillomavirus 6	NIBSC-UK-EN63QG	None
Human papillomavirus 11	NIBSC-UK-EN63QG	None
Human papillomavirus type 58	NIBSC-UK-EN63QG	None
Human papillomavirus type 33	NIBSC-UK-EN63QG	None
Neisseria meningitidis	ATCC 13090	None
Herpes Simplex Virus 1	ATCC VR-1778	None
Herpes Simplex Virus 2	Zeptometrix 0810217CF	None
Treponema pallidum	ATCC BAA-2642SD	None
Chlamydia trachomatis	Zeptometrix 0801775	None
Neisseria gonorrhoeae	ATCC 19424	None
Ureaplasma urealyticum	ATCC 27618	None
Ureaplasma parvum	ATCC 27815	None
Mycoplasma hominis	ATCC 27545-TTR	None
Mycoplasma genitalium	ATCC 33530D	None

10.5. Interferences

The ability of endogenous or exogenous substances that could be present in research samples (or introduced during sample collection and handling) to interfere with accurate detection of analytes was evaluated with select direct testing on the ***MarinaBiolab Women Health Panel PCR Kit*** and extrapolated from the interference evaluation of the ***MarinaBiolab Women Health Panel PCR Kit***.

Potentially interfering substances were evaluated using contrived samples spiked with substance. Results from samples containing a substance were compared to results from control samples without substance. The substances tested included endogenous substances that may be found in sample at normal or elevated levels (e.g., blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications, various swabs and transport media for sample collection, and substances used to clean, decontaminate, or disinfect work areas. Each substance was added to contrived samples containing representative organisms at concentrations near (3x) LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in research sample and each sample was tested in triplicate.

None of the substances were shown to interfere with the ***MarinaBiolab Women Health Panel PCR Kit***.

Table 15. Evaluation of potentially interfering substances on the ***MarinaBiolab Women Health Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Human Blood	10% v/v	No Interference
Human Mucus	1 swab/mL sample	No Interference
Human Genomic DNA	20 ng/µL	No Interference
Competitive Microorganisms		
Haemophilus ducreyi	1.0E+06 CFU/mL	No Interference
Bacterial Vaginosis Associated Bacteria 2	1.0E+06 CFU/mL	No Interference
Fannyhessea vaginae	1.0E+06 CFU/mL	No Interference
Candida albicans	1.0E+06 CFU/mL	No Interference
Candida glabrata	1.0E+06 CFU/mL	No Interference
Candida parapsilosis	1.0E+06 CFU/mL	No Interference
Escherichia coli	1.0E+06 CFU/mL	No Interference
Streptococcus agalactiae	1.0E+06 CFU/mL	No Interference
Prevotella bivia	1.0E+06 CFU/mL	No Interference
Lactobacillus gasseri	1.0E+06 CFU/mL	No Interference
Lactobacillus iners	1.0E+06 CFU/mL	No Interference
Lactobacillus crispatus	1.0E+06 CFU/mL	No Interference
Megasphaera phylotypes 1	1.0E+06 CFU/mL	No Interference
Megasphaera phylotypes 2	1.0E+06 CFU/mL	No Interference

Staphylococcus aureus	1.0E+06 CFU/mL	No Interference
Mobiluncus curtisii	1.0E+06 CFU/mL	No Interference
Mobiluncus mulieris	1.0E+06 CFU/mL	No Interference
Gardnerella vaginalis	1.0E+06 CFU/mL	No Interference
Enterococcus faecalis	1.0E+06 CFU/mL	No Interference
Lactobacillus jensenii	1.0E+06 CFU/mL	No Interference
Candida tropicalis	1.0E+06 CFU/mL	No Interference
Trichomonas vaginalis	1.0E+06 CFU/mL	No Interference
Exogenous Substances		
K-Y Personal Lubricant Jelly	1% v/v	No Interference
Ortho Options Gynol II Extra Strength Vaginal Contraceptive Jelly	1% v/v	No Interference
Azithromycin	1.8 mg/mL	No Interference
Vagisil Creme Maximum Strength	1% w/v	No Interference
Aspirin	40 mg/mL	No Interference
K-Y Personal Lubricant Jelly	1% v/v	No Interference
Specimen Collection Materials		
Copan Liquid Amies Elution Swab (ESwab®)	N/A	No Interference

11. TROUBLESHOOTING

Problem	Cause	Solution
Target-specific and/or IC signals are detected in the Negative Control well.	Contamination from the environment, contamination of extraction and/or qPCR reagents, or well-to-well cross contamination. The signal is not true target amplification, but background curves generated by the software of the qPCR instrument.	Repeat the qPCR with new reagents. Follow the general rules of GLP in a PCR lab (e.g., Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Ensure that filters tips are used during the procedure and changed between samples.). It is recommended to set up the qPCR reactions in a separate area, where no RNA/DNA is handled and with equipment designated for pre-PCR activities. Ignore the Cq value of NTC if the amplification curve looks not real but background noise. If the problem persists, contact Technical Support.
No IC signal is detected, but target-specific signal is detected in sample wells.	A high copy number of target nucleic acid exists in samples, resulting in preferential amplification of the target-specific nucleic acid.	No action is required. The result is considered positive.
The Positive Control did not meet the criteria set for acceptable values of the kit. The assay is invalid.	Positive Control was not stored at the recommended conditions. Kit shelf-life expired.	Check the kit label for storage conditions and expiration date. Replace the Positive Control. Use a new kit if necessary.
High Cq values observed for repeated samples.	Frozen samples were not mixed properly after thawing. Degraded nucleic acids.	Make sure, thaw frozen samples with mild agitation to ensure thorough mixing. Ensure that samples are stored correctly and not subjected to multiple freeze-thaw cycles
Target-specific and/or IC signal detected after 38 Cycles in Positive Control.	Incorrect qPCR set-up or the kit reagents may have been compromised (e.g., improper storage or more than 15 freeze-thaw cycles).	Replace the control. If the problem persists, contact Technical Support.
No target-specific and IC signal is detected in sample wells.	Sampling, extraction, or inhibition problem.	Dilute the nucleic acid isolate 1/10 and repeat the qPCR. If the diluted sample does not give a positive result in the IC channel, request for a new sample and repeat the NA extraction. Repeat the NA extraction and the qPCR. Request for a new sample, repeat the NA extraction and the qPCR. If the problem persists, contact Technical Support.

12. EXPLANATION of SYMBOLS

Symbol	Title of Symbol	Symbol	Title of Symbol
	Research Use Only		Use-by date
	Manufacturer		Batch code
	Negative control		Non-sterile
	Positive control		Consult instructions for use or consult electronic instructions for use
	Control		Caution
	Temperature limit		Catalogue number
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Keep dry		Keep upright
	Contains sufficient for <n> tests		Protect from heat and radioactive sources

Custom care and technical support

Tel: +1 510 579-5802

e-mail customer care: accounting@marinabiolab.com

e-mail Technical Support: rd@marinabiolab.com

MarinaBiolab LTD.

Address: Hürriyet Mah. Erler So. No: 4/1A Nexus No: 13 Kartal/Istanbul/Turkey

For research use only (RUO)! Not for use in diagnostic procedures.