RUO

INSTRUCTION FOR USE

Pre-Plated Urinary Tract Infection Plus Panel PCR Kit

For Research Use Only

Σ 96

REF PP-UTI Plus 003

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Document Revision History

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Rev.00_June 20, 2024	First Release
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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, this test kit is not intended for the diagnosis of infectious diseases in animals.

The ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** is a multiplex, qualitative Real-Time Polymerase Chain Reaction (qPCR) test intended for the simultaneous detection and identification of multiple pathogenic nucleic acids in research samples. The kit enables qPCR results in less than one hour. It is designed to detect gene sequences from the following organisms:

Targets	
Escherichia coli	*Staphylococcus aureus*
Streptococcus agalactiae	*Ureaplasma urealyticum*
Klebsiella oxytoca/michiganensis	*Citrobacter koseri*
Staphylococcus saprophyticus	*Candida albicans*
Serratia marcescens	*Candida glabrata*
Proteus mirabilis	*Candida parapsilosis*
Mycoplasma hominis	*Bacteroides fragilis*
Enterococcus spp.	*Candida tropicalis*
Enterobacter cloacae	*Candida krusei*
Pseudomonas aeruginosa	*Mycoplasma genitalium*
Citrobacter freundii/braakii	Klebsiella pneumoniae carbapenem resistance gene (KPC)
Klebsiella aerogenes	Quinolone resistance gene (QNR)
Klebsiella pneumoniae	New Delhi metallo-beta-lactamase resistance gene (NDM)
Morganella morganii	Methicillin resistance genes (mecA/C)
Prevotella bivia	Vancomycin resistance gene (VanA)
Streptococcus pyogenes	Vancomycin resistance gene (VanB)
Candida dubliniensis	Sulfonamide resistant genes (Sul1/2)
Acinetobacter baumannii	Cefotaxime-M-b-lactamase resistance gene (CTX)
Staphylococcus epidermidis	Trimethoprim resistance gene (DfrA)
Controls	
Human RNase P (IC)	
Bacillus atrophaeus (EC)	

2. PRINCIPLE of the PROCEDURE

DNA target regions are amplified using real-time PCR instruments, along with the specific primer and probe sets provided in the kit. During amplification, each probe binds to 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 cleaves the probe, separating the reporter dye from the quencher and generating a fluorescent signal. With each cycle, more reporter dye molecules are released, resulting in an increase in fluorescence intensity. Fluorescence is measured at each cycle by the real-time PCR instrument. Probes labeled with distinct fluorophores are used to detect specific amplicons derived from both the target sequences and the internal control. The PCR instrument monitors the fluorescence signals in real time and interprets the data to provide a qualitative result for each target. A positive result for the presence of target DNA is indicated by the appearance of a real-time PCR amplification curve and a corresponding Cq (Quantification Cycle) value.

3. KIT COMPONENTS

The ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** consists of three main components:

1. qPCR Enzyme, Buffer, Forward, Reverse and Probe Mix (Pre-Plated UTI Plus Mix 1-11)
2. A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below (PC-UTI Plus)
3. DNase/RNase-Free Water (NTC-UTI Plus)

The components of the kit are provided in Table 1-2.

Table 1. Kit components.

Component	Description	Quantity x Volume 96 rxn PP-UTI Plus 003
Pre-Plated UTI Plus Mix 1-8 **Strip 1**	Ready-to-use mix for qPCR	96 Strips (15 µL)
Pre-Plated UTI Plus Mix 9-11 **Strip 2**	Ready-to-use mix for qPCR	96 Strips (15 µL)
PC-UTI Plus	A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below	2 x 400 µL
NTC-UTI Plus	DNase/RNase-Free Water	2 x 400 µL
PC-UTI Plus Pre-Mix	A mixture of non-infectious cDNA and DNA from artificial samples + Oligo + Master Mix	1 x 200 µL
NTC-UTI Plus Pre-Mix	DNase/RNase-Free Water + Oliog + Master Mix	1 x 200 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
UTI Plus Oligo Mix 1	*Escherichia coli*	**FAM**
	Streptococcus agalactiae	**HEX/VIC/JOE**
	Klebsiella oxytoca/michiganensis	**ROX/Texas Red**
	Human RNase P (IC)	**CY5**
UTI Plus Oligo Mix 2	*Staphylococcus saprophyticus*	**FAM**
	Serratia marcescens	**HEX/VIC/JOE**
	Proteus mirabilis	**ROX/Texas Red**
	Mycoplasma hominis	**CY5**
	Enterococcus spp.	**FAM**
	Enterobacter cloacae	**HEX/VIC/JOE**

UTI Plus Oligo Mix 3	*Pseudomonas aeruginosa*	ROX/Texas Red
	Citrobacter freundii/braakii	CY5

UTI Plus Oligo Mix 4	*Klebsiella aerogenes*	FAM
	Klebsiella pneumoniae	HEX/VIC/JOE
	Morganella morganii	ROX/Texas Red
	Prevotella bivia	CY5
UTI Plus Oligo Mix 5	*Streptococcus pyogenes*	FAM
	Candida dubliniensis	HEX/VIC/JOE
	Acinetobacter baumannii	ROX/Texas Red
	Staphylococcus epidermidis	CY5
UTI Plus Oligo Mix 6	*Staphylococcus aureus*	FAM
	-	HEX/VIC/JOE
	Ureaplasma urealyticum	ROX/Texas Red
	Citrobacter koseri	CY5
UTI Plus Oligo Mix 7	*Candida albicans*	FAM
	Candida glabrata	HEX/VIC/JOE
	Candida parapsilosis	ROX/Texas Red
	Bacteroides fragilis	CY5
UTI Plus Oligo Mix 8	*Candida tropicalis*	FAM
	Candida krusei	HEX/VIC/JOE
	Mycoplasma genitalium	ROX/Texas Red
	Bacillus atrophaeus (EC)	CY5
UTI Plus Oligo Mix 9	KPC	FAM
	QNR	HEX/VIC/JOE
	NDM	ROX/Texas Red
	-	CY5
UTI Plus Oligo Mix 10	mecA/C	FAM
	VanA	HEX/VIC/JOE
	VanB	ROX/Texas Red
	-	CY5
UTI Plus Oligo Mix 11	Sul	FAM
	CTX	HEX/VIC/JOE
	DfrA	ROX/Texas Red
	-	CY5

The oligonucleotide set targeting the human *RNase P* (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 to evaluate 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 with Maestro software v1.1 and consumables

5. WARNING and PRECAUTIONS

- The ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** is intended for research use only and should be used by professionally trained, qualified personnel. All procedures should be performed in accordance with Good Laboratory Practices (GLP).
- Biological material used for nucleic acid extraction should be handled as potentially infectious. Appropriate safety precautions are recommended when handling biological material (e.g., do not pipet by mouth; wear disposable gloves; disinfect hands after completing the test).
- Biological material should be inactivated before disposal (e.g., autoclaving). Disposable items should be autoclaved or incinerated after use.
- In the event of a spill involving potentially infectious materials, the spill should be immediately absorbed with paper tissue, and the affected area should be disinfected using a suitable standard disinfectant or 70% alcohol. Materials used for cleaning spills, including gloves, should be inactivated before disposal (e.g., autoclaving).
- Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state, and local regulations.
- To avoid microbial contamination of reagents during aliquoting, it is recommended to use sterile, single-use pipettes and tips. Reagents that appear cloudy or show signs of microbial contamination should not be used.
- The kit should be stored away from nucleic acid sources and PCR amplicons to prevent contamination.
- Always check the expiration date on the kit. Do not use expired or improperly stored kits.
- Components in the kit should not be mixed with components from different lot numbers or from different manufacturers, even if they contain the same components.
- The kit components should be gently mixed before use by shaking.
- A common issue with PCR-based assays is false positive results caused by contamination from PCR amplicons. To minimize the risk of amplicon contamination:
    - Ensure separate work areas with dedicated apparatus are available for each stage of the procedure.
    - Do not open reaction tubes/plates post-amplification to avoid contamination with amplicons.
    - Discard used tubes/plates immediately in a biohazard container after completing the run.
    - Minimize handling of tubes/plates after testing.
    - Change gloves after handling used tubes/plates.

6. HANDLING, STORAGE, and STABILITY

- The ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** is shipped on dry ice. If any component is not frozen upon arrival or if the outer packaging has been compromised during shipment, please contact **MarinaBiolab** or the local distributor immediately.
- Upon arrival, all components should be stored between -25°C and -15°C.
- Repeated freezing and thawing of the kit components may reduce detection quality. The kit can withstand up to 15 freeze/thaw cycles without impacting performance.
- When stored under the specified conditions, the kit remains stable until the expiration date printed on the package. The expiration date is 12 months from the date of manufacture.
- All components must be thawed at ambient temperature for at least 30 minutes before use.
- It is recommended to keep all components on ice when preparing the assay mixes.
- The primer and probe mixes contain fluorophore-labeled probes and should be protected from direct sunlight and prolonged exposure to ambient light.
- Do not use expired or improperly stored components.

7. TEST PROCEDURE

7.1. Sample Preparation and Nucleic Acid Extraction

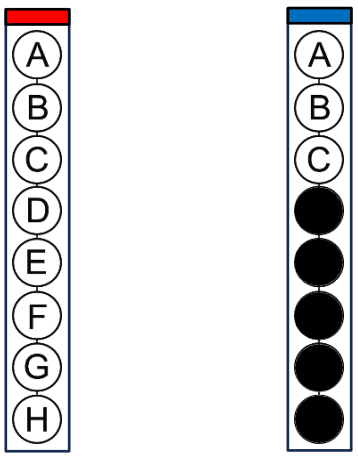
Samples intended for nucleic acid isolation must be collected using appropriate cell collection systems. The performance of the kit is highly dependent on both the quantity and quality of the extracted nucleic acid. Ensure that the extraction method used is compatible with real-time PCR technology.

If the laboratory's established standard protocol is used for nucleic acid isolation, it must be validated by the end user.

For frozen samples or previously extracted nucleic acid, thaw only the amount required for testing on the same day. Avoid multiple freeze/thaw cycles, as these can compromise nucleic acid integrity. For best results, use the nucleic acid immediately after thawing.

7.2. PCR Reaction Preparation and Processing

- Determine the number of reactions needed and prepare a PCR plate layout accordingly.
- The plate layout should include the following:
    - Reactions for each test sample and extraction negative control.
    - PCR control reactions:
        - Positive Control (provided in the kit)
        - Negative (No Template) Control (NTC) (provided in the kit)
- Completely thaw all components at room temperature for at least 30 minutes prior to use.
- When they thaw, vortex and **spin down** briefly the components and place them on cold block during the whole test procedure.
- Use 2 strips (Strip 1 and Strip 2) for each sample or control.
- The orientations of Strip 1 and 2 should be as shown below.



Strip 1 **Strip 2**

- Open carefully the strips (gently open it from the side way, DO NOT PEEL OFF THE LID) and add 5 µL of the isolated sample or control to the corresponding wells or 20 µL of Pre-Mix PC or NTC to an empty well..
- The final reaction mix volume is 20 µL.
- Re-cap the strips and **spin down** for 5 seconds.
- Insert strips into the real-time PCR instrument and amplify according to the following PCR profile.

For each run, use one well of PC-Mix and one well of NTC-Mix as shown in the diagram below.

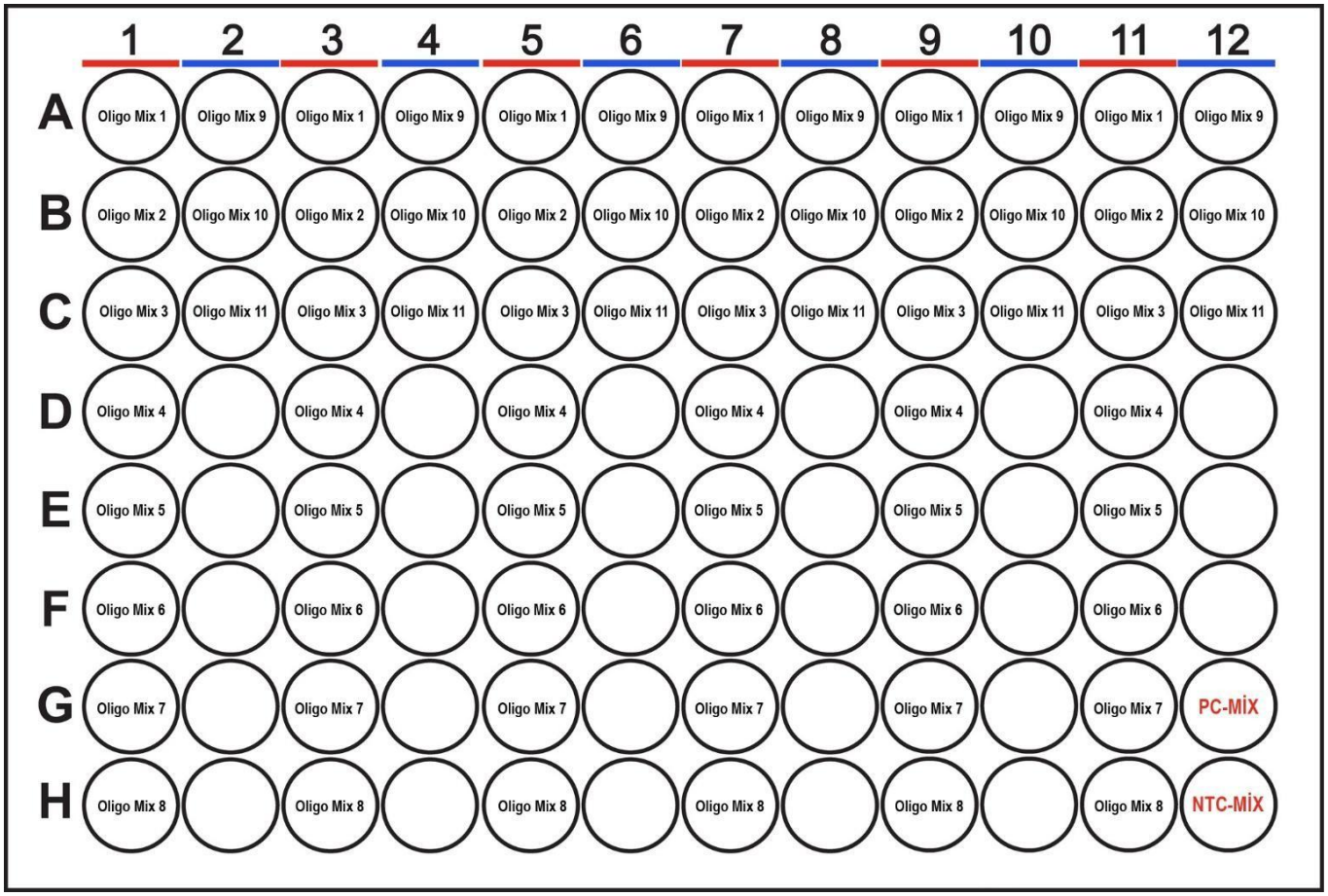


Table 3. Amplification profile.

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

8. INTERPRETATION OF RESULTS

MarinaBiolab Pre-Plated Urinary Tract Infection Plus 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

Configure the following instrument settings before evaluating the results.

Table 4. Instrument-specific settings.

Instrument	Threshold Level	Other Settings
CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad)	500 RFU	-
QuantStudio™ 5, 7 and 12K (Applied Biosystems™)	Auto	-

The shape of the amplification curves should be evaluated. If the instrument's software assigns a Cq value to a sample and the curve is sigmoidal, the Cq value can be used in the final assessment. *Non-sigmoidal curves should be recorded as negative.*

A result is considered positive if the Cq value is ≤35, or as determined by your laboratory's protocols.

8.2. Overall Validity of Detection

Table 5. 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 sample results, we recommend verifying the validity of the real-time PCR test. For each run, please confirm that the Positive and Negative controls performed as expected, based on the following criteria:

Table 6. 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	Proceed with the interpretation of sample results.

Any of them is Negative	Not considered	INVALID	Contact the manufacturer, replenish the reagents, and repeat the reaction.
Not considered	Any of them is Positive	INVALID	Repeat the analysis, ensuring to follow the 'Warnings and Precautions' outlined in the IFU.

If any control fails to perform as described above, the run is considered invalid and must be repeated. If the issue persists, contact the manufacturer.

If all controls perform as expected, proceed with the interpretation of the results.

8.3. Interpretation of Unknown Specimen Results

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

Table 7. Interpretation of unknown specimen results for DNA pathogens.

DNA Pathogens	Internal Control (*RNase P*)	External Control (*Bacillus atrophaeus*)	Results	Interpretation
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Invalid	Repeat the test by re-extracting the sample. If the result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Positive (+) (Cq<35)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Invalid	Repeat the test by re-extracting the sample. If the result remains invalid, consider collecting a new sample.

9. ASSAY LIMITATIONS

- The ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** is intended for use only by professionally trained and qualified staff.
- A false negative result may occur if the specimen is improperly collected, transported, or handled. False negatives can also occur if amplification inhibitors are present in the specimen or if insufficient numbers of organisms are present.
- Spontaneous mutations within the target sequences may result in failure to detect the target. While the test design mitigates this risk, if target detection failure is anticipated, it is recommended to test the specimen with a different assay that targets other sequences in the genome.
- There is a risk of false positive results due to cross-contamination by target viruses and/or bacteria, their nucleic acids or amplified products, or from non-specific signals in the assay. Proper handling of consumables, as outlined in the Warnings and Precautions section, is crucial to minimize this risk.
- This assay is qualitative and does not provide a quantitative assessment of the detected organism's concentration.
- All instruments (e.g., pipettes, real-time PCR cyclers) must be calibrated according to the manufacturer's instructions.

10. PERFORMANCE CHARACTERISTICS

10.1. Analytical Sensitivity (Limit of Detection, LoD)

The limit of detection (LoD) was defined as the concentration at which the test produces a positive result more than 95% of the time. Serial dilutions of the strains were tested, and the initial tentative LoD was confirmed with twenty (20) replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, an additional twenty (20) replicates were performed at the next lower concentration until a detection rate of ≤95% was achieved.

For nucleic acid extraction, a simulated research matrix was spiked with strains and processed using the Automatic Nucleic Acids Extraction Instrument. Testing was carried out on the CFX96 Touch™ (Bio-Rad) Real-Time PCR system. The confirmed LoDs for the strains tested, along with the corresponding LoDs for the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** reportable targets, are presented in Table 8 below.

Table 8. Summary of LoD study results.

Analyte	Isolate ID/Source	LoD Concentration (copies/mL)	Detected/Total
Escherichia coli	ATCC 25922	3.5E+01 copies/mL	**20/20** 100%
Streptococcus agalactiae	ATCC 12386	6.7E+01 copies/mL	**19/20** 95%
Klebsiella oxytoca	ATCC 700324	2.6E+01 copies/mL	**20/20** 100%
Klebsiella michiganensis	ATCC 8724	3.4E+01 copies/mL	**20/20** 100%
Staphylococcus saprophyticus	Zeptometrix 0804014	5.7E+01 copies/mL	**20/20** 100%
Serratia marcescens	ATCC 29021	2.1E+02 copies/mL	**20/20** 100%
Proteus mirabilis	Zeptometrix 0801544	2.1E+02 copies/mL	**20/20** 100%
Mycoplasma hominis	ATCC 27545-TTR	1.1E+02 copies/mL	**20/20** 100%
Enterococcus spp.	ATCC BAA-2127	4.5E+01 copies/mL	**20/20** 100%
Enterobacter cloacae	Zeptometrix 0801830	7.4E+01 copies/mL	**19/20** 95%
Pseudomonas aeruginosa	ATCC 27853	6.7E+02 copies/mL	**20/20** 100%
Citrobacter freundii	Zeptometrix 0801563	4.2E+01 copies/mL	**20/20** 100%

Citrobacter braakii	Zeptometrix 0804366	3.1E+01 copies/mL	**20/20** 100%
Klebsiella aerogenes	ATCC 13048	2.4E+02 copies/mL	**20/20** 100%
Klebsiella pneumoniae	NCTC 13465	3.0E+01 copies/mL	**20/20** 100%
Morganella morganii	Zeptometrix 0804010	4.8E+01 copies/mL	**20/20** 100%
Prevotella bivia	Zeptometrix 0801756	8.9+01 copies/mL	**20/20** 100%
Streptococcus pyogenes	Zeptometrix 0801512	3.5E+01 copies/mL	**20/20** 100%
Candida dubliniensis	Zeptometrix 0801915	1.5E+02 copies/mL	**20/20** 100%
Acinetobacter baumannii	ATCC 19606	1.7E+02 copies/mL	**20/20** 100%
Staphylococcus epidermidis	Zeptometrix 0804281	4.9E+01 copies/mL	**20/20** 100%
Staphylococcus aureus	ATCC 10832	5.5E+01 copies/mL	**20/20** 100%
Ureaplasma urealyticum	ATCC 27618	5.0E+01 copies/mL	**20/20** 100%
Citrobacter koseri	Zeptometrix 0801745	7.9E+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%
Bacteroides fragilis	Zeptometrix 0801583	6.4E+01 copies/mL	**20/20** 100%
Candida tropicalis	ATCC 750	5.7E+01 copies/mL	**20/20** 100%
Candida krusei	ATCC 2159	6.8E+01 copies/mL	**20/20** 100%
Mycoplasma genitalium	ATCC 33530D	4.0E+01 copies/mL	**19/20** 95%

KPC	Zeptometrix NATPPQ-BIO	1.4E+02 copies/mL	**20/20** 100%
QNR	ATCC BAA-2728	1.1E+02 copies/mL	**20/20** 100%
NDM	Zeptometrix NATPPQ-BIO	8.5E+01 copies/mL	**20/20** 100%
mecA	ATCC BAA-2094	9.9E+01 copies/mL	**20/20** 100%
mecC	ATCC BAA-2313	8.5E+01 copies/mL	**20/20** 100%
VanA	Zeptometrix 0801892	7.4E+01 copies/mL	**20/20** 100%
VanB	Zeptometrix 0801953	1.7E+02 copies/mL	**20/20** 100%
Sul1	ATCC BAA-3035	1.6E+02 copies/mL	**20/20** 100%
Sul2	ATCC BAA-2894	1.4E+02 copies/mL	**20/20** 100%
CTX	Zeptometrix NATPPQ-BIO	9.6E+01 copies/mL	**20/20** 100%
DfrA	ATCC BAA-3041	6.7E+01 copies/mL	**20/20** 100%

10.2. Device Equivalence Study

A device equivalence study was conducted to assess the differences in results obtained using the kit across various instruments. For this purpose, the same LoD determination study was repeated using 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 results were obtained at the 1x LoD concentration level of the targets in the device equivalence study across the different instruments.

10.3. Analytical Reactivity (Inclusivity)

10.3.1. In-Slico Analytical Reactivity

A BLAST search of the oligonucleotides was conducted on the genome sequences of *Escherichia coli, Streptococcus agalactiae, Klebsiella oxytoca, Klebsiella michiganensis, Staphylococcus saprophyticus, Serratia marcescens, Proteus mirabilis, Mycoplasma hominis, Enterococcus spp., Enterobacter cloacae, Pseudomonas aeruginosa, Citrobacter freundii, Citrobacter braakii, Klebsiella aerogenes, Klebsiella pneumoniae, Morganella morganii, Prevotella bivia, Streptococcus pyogenes, Candida dubliniensis, Acinetobacter baumannii, Staphylococcus epidermidis, Staphylococcus aureus, Ureaplasma urealyticum, Citrobacter koseri, Candida*

albicans, Candida glabrata, Candida parapsilosis, Bacteroides fragilis, Candida tropicalis, Candida krusei, Mycoplasma genitalium, KPC, QNR, NDM, mecA, mecC, VanA, VanB, Sul1, Sul2, CTX, and DfrA using the Primer-BLAST tool on the NCBI database.

The aggregated results of all in-silico analyses performed using the NCBI database are provided in the table below. The melting temperatures (Tm) of the oligonucleotide sequences with a 1-base mismatch remain higher than the annealing temperature specified in the PCR cycle parameters of the kit. Therefore, single base mismatches in the sequences are not expected to impact the inclusivity of the test.

Table 9. 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
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%
Klebsiella oxytoca	Sense Primer	150	99.74%	0.26%	0.00%	0.00%
Klebsiella oxytoca	Antisense Primer	158	99.56%	0.44%	0.00%	0.00%
Klebsiella oxytoca	Hydrolysis Probe	158	99.83%	0.27%	0.00%	0.00%
Klebsiella michiganensis	Sense Primer	56	100.00%	0.00%	0.00%	0.00%
Klebsiella michiganensis	Antisense Primer	56	100.00%	0.00%	0.00%	0.00%
Klebsiella michiganensis	Hydrolysis Probe	55	100.00%	0.00%	0.00%	0.00%
Staphylococcus saprophyticus	Sense Primer	26	99.52%	0.48%	0.00%	0.00%
Staphylococcus saprophyticus	Antisense Primer	26	97.52%	2.48%	0.00%	0.00%
Staphylococcus saprophyticus	Hydrolysis Probe	26	99.74%	0.26%	0.00%	0.00%
Serratia marcescens	Sense Primer	296	99.80%	0.20%	0.00%	0.00%
Serratia marcescens	Antisense Primer	296	99.80%	0.20%	0.00%	0.00%
Serratia marcescens	Hydrolysis Probe	292	99.82%	0.18%	0.00%	0.00%
Proteus mirabilis	Sense Primer	164	100.00%	0.00%	0.00%	0.00%
Proteus mirabilis	Antisense Primer	164	100.00%	0.00%	0.00%	0.00%
Proteus mirabilis	Hydrolysis Probe	160	99.80%	0.20%	0.00%	0.00%
Mycoplasma hominis	Sense Primer	48	100.00%	0.00%	0.00%	0.00%
Mycoplasma hominis	Antisense Primer	48	100.00%	0.00%	0.00%	0.00%

Mycoplasma hominis	Hydrolysis Probe	48	100.00%	0.00%	0.00%	0.00%
Enterococcus spp.	Sense Primer	1121	98.68%	1.32%	0.00%	0.00%
Enterococcus spp.	Antisense Primer	1121	98.68%	1.32%	0.00%	0.00%
Enterococcus spp.	Hydrolysis Probe	1110	98.46%	1.54%	0.00%	0.00%
Enterobacter cloacae	Sense Primer	683	99.63%	0.37%	0.00%	0.00%
Enterobacter cloacae	Antisense Primer	669	99.12%	0.88%	0.00%	0.00%
Enterobacter cloacae	Hydrolysis Probe	669	99.82%	0.18%	0.00%	0.00%
Pseudomonas aeruginosa	Sense Primer	1.162	99.75%	0.25%	0.00%	0.00%
Pseudomonas aeruginosa	Antisense Primer	1.167	99.79%	0.21%	0.00%	0.00%
Pseudomonas aeruginosa	Hydrolysis Probe	1.167	99.84%	0.16%	0.00%	0.00%
Citrobacter freundii	Sense Primer	175	99.70%	0.30%	0.00%	0.00%
Citrobacter freundii	Antisense Primer	175	99.70%	0.30%	0.00%	0.00%
Citrobacter freundii	Hydrolysis Probe	168	99.90%	0.10%	0.00%	0.00%
Citrobacter braakii	Sense Primer	85	100.00%	0.00%	0.00%	0.00%
Citrobacter braakii	Antisense Primer	85	100.00%	0.00%	0.00%	0.00%
Citrobacter braakii	Hydrolysis Probe	82	100.00%	0.00%	0.00%	0.00%
Klebsiella aerogenes	Sense Primer	83	98.52%	1.48%	0.00%	0.00%
Klebsiella aerogenes	Antisense Primer	82	97.11%	2.89%	0.00%	0.00%
Klebsiella aerogenes	Hydrolysis Probe	82	96.85%	3.15%	0.00%	0.00%
Klebsiella pneumoniae	Sense Primer	2.816	100.00%	0.00%	0.00%	0.00%
Klebsiella pneumoniae	Antisense Primer	2.711	100.00%	0.00%	0.00%	0.00%
Klebsiella pneumoniae	Hydrolysis Probe	2.711	99.66%	0.34%	0.00%	0.00%
Morganella morganii	Sense Primer	81	99.84%	0.16%	0.00%	0.00%
Morganella morganii	Antisense Primer	81	99.84%	0.16%	0.00%	0.00%
Morganella morganii	Hydrolysis Probe	80	99.52%	0.48%	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%
Streptococcus pyogenes	Sense Primer	389	100.00%	0.00%	0.00%	0.00%
Streptococcus pyogenes	Antisense Primer	389	99.89%	0.11%	0.00%	0.00%
Streptococcus pyogenes	Hydrolysis Probe	389	99.97%	0.03%	0.00%	0.00%
Candida dubliniensis	Sense Primer	1070	99.89%	0.11%	0.00%	0.00%

Candida dubliniensis	Antisense Primer	1070	99.83%	0.17%	0.00%	0.00%
Candida dubliniensis	Hydrolysis Probe	1073	99.65%	0.35%	0.00%	0.00%
Acinetobacter baumannii	Sense Primer	1.703	99.35%	0.65%	0.00%	0.00%
Acinetobacter baumannii	Antisense Primer	1.701	99.89%	0.21%	0.00%	0.00%
Acinetobacter baumannii	Hydrolysis Probe	1.701	99.47%	0.53%	0.00%	0.00%
Staphylococcus epidermidis	Sense Primer	230	100%	0.00%	0.00%	0.00%
Staphylococcus epidermidis	Antisense Primer	232	99.74%	0.26%	0.00%	0.00%
Staphylococcus epidermidis	Hydrolysis Probe	232	100%	0.00%	0.00%	0.00%
Staphylococcus aureus	Sense Primer	2.491	99.65%	0.35%	0.00%	0.00%
Staphylococcus aureus	Antisense Primer	2.703	99.74%	0.26%	0.00%	0.00%
Staphylococcus aureus	Hydrolysis Probe	2.703	99.62%	0.38%	0.00%	0.00%
Ureaplasma urealyticum	Sense Primer	90	99.90%	0.10%	0.00%	0.00%
Ureaplasma urealyticum	Antisense Primer	90	99.90%	0.10%	0.00%	0.00%
Ureaplasma urealyticum	Hydrolysis Probe	88	99.90%	0.10%	0.00%	0.00%
Citrobacter koseri	Sense Primer	226	99.95%	0.05%	0.00%	0.00%
Citrobacter koseri	Antisense Primer	236	100.00%	0.00%	0.00%	0.00%
Citrobacter koseri	Hydrolysis Probe	236	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 krusei	Sense Primer	1.415	100%	0.00%	0.00%	0.00%
Candida krusei	Antisense Primer	1.415	100%	0.00%	0.00%	0.00%
Candida krusei	Hydrolysis Probe	1.415	100%	0.00%	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%
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%

Bacteroides fragilis	Sense Primer	597	99.20%	0.80%	0.00%	0.00%
Bacteroides fragilis	Antisense Primer	597	99.20%	0.80%	0.00%	0.00%
Bacteroides fragilis	Hydrolysis Probe	590	99.05%	0.80%	0.05%	0.00%
Mycoplasma genitalium	Sense Primer	50	100.00%	0.00%	0.00%	0.00%
Mycoplasma genitalium	Antisense Primer	50	100.00%	0.00%	0.00%	0.00%
Mycoplasma genitalium	Hydrolysis Probe	48	100.00%	0.00%	0.00%	0.00%
KPC	Sense Primer	24152	98.25%	1.50%	0.25%	0.00%
KPC	Antisense Primer	24152	98.25%	1.50%	0.25%	0.00%
KPC	Hydrolysis Probe	22468	98.13%	1.43%	0.44%	0.00%
QNR	Sense Primer	1583	99.12%	0.88%	0.00%	0.00%
QNR	Antisense Primer	1583	99.12%	0.88%	0.00%	0.00%
QNR	Hydrolysis Probe	1488	99.00%	0.73%	0.27%	0.00%
NDM	Sense Primer	2465	98.78%	1.20%	0.02%	0.00%
NDM	Antisense Primer	2465	98.78%	1.20%	0.02%	0.00%
NDM	Hydrolysis Probe	2385	98.68%	1.10%	0.22%	0.00%
mecA/C	Sense Primer	1.981	99.72%	0.28%	0.00%	0.00%
mecA/C	Antisense Primer	1.993	97.23%	2.77%	0.00%	0.00%
mecA/C	Hydrolysis Probe	1.993	99.69%	0.31%	0.00%	0.00%
VanA	Sense Primer	186	100.00%	0.00%	0.00%	0.00%
VanA	Antisense Primer	186	100.00%	0.00%	0.00%	0.00%
VanA	Hydrolysis Probe	186	100.00%	0.00%	0.00%	0.00%
VanB	Sense Primer	8669	98.75%	1.00%	0.25%	0.00%
VanB	Antisense Primer	8669	98.75%	1.00%	0.25%	0.00%
VanB	Hydrolysis Probe	8453	98.54%	1.16%	0.30%	0.00%
Sul1	Sense Primer	6428	99.64%	0.36%	0.00%	0.00%
Sul1	Antisense Primer	6428	99.64%	0.36%	0.00%	0.00%
Sul1	Hydrolysis Probe	6432	99.52%	0.48%	0.00%	0.00%
Sul2	Sense Primer	5435	99.80%	0.20%	0.00%	0.00%
Sul2	Antisense Primer	5435	99.80%	0.20%	0.00%	0.00%
Sul2	Hydrolysis Probe	5400	99.72%	0.28%	0.00%	0.00%
DfrA	Sense Primer	1946	99.90%	0.10%	0.00%	0.00%
DfrA	Antisense Primer	1946	99.90%	0.10%	0.00%	0.00%

DfrA	Hydrolysis Probe	1940	99.90%	0.10%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** was demonstrated using a comprehensive panel that represents the temporal, evolutionary, and geographic diversity of each target organism.

Each sample was tested in triplicate with the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** 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 3-fold higher were evaluated.

The individual strains and the concentrations at which positive test results were obtained for all three replicates are presented by target organisms in Table 10 below.

Table 10. Results of the wet inclusivity test.

Variant/Type/Subtype/Lineage/Genotype/Species	Isolate ID/Source	xLoD Detected
Escherichia coli	ATCC 25922	1x
Streptococcus agalactiae	ATCC 12386	1x
Klebsiella oxytoca	ATCC 700324	1x
Klebsiella michiganensis	ATCC 8724	1x
Staphylococcus saprophyticus	Zeptometrix 0804014	1x
Serratia marcescens	ATCC 29021	1x
Proteus mirabilis	Zeptometrix 0801544	1x
Mycoplasma hominis	ATCC 27545-TTR	1x
Enterococcus spp.	ATCC BAA-2127	1x
Enterobacter cloacae	Zeptometrix 0801830	1x
Pseudomonas aeruginosa	ATCC 27853	1x
Citrobacter freundii	Zeptometrix 0801563	1x
Citrobacter braakii	Zeptometrix 0804366	1x
Klebsiella aerogenes	ATCC 13048	1x
Klebsiella pneumoniae	NCTC 13465	1x
Morganella morganii	Zeptometrix 0804010	1x
Prevotella bivia	Zeptometrix 0801756	1x
Streptococcus pyogenes	Zeptometrix 0801512	1x
Candida dubliniensis	Zeptometrix 0801915	1x
Acinetobacter baumannii	ATCC 19606	1x

Staphylococcus epidermidis	Zeptometrix 0804281	1x
Staphylococcus aureus	ATCC 10832	1x
Ureaplasma urealyticum	ATCC 27618	1x
Citrobacter koseri	Zeptometrix 0801745	1x
Candida albicans	ATCC 10231	1x
Candida glabrata	ATCC 90030	1x
Candida parapsilosis	ATCC 22019	1x
Bacteroides fragilis	Zeptometrix 0801583	1x
Candida tropicalis	ATCC 750	1x
Candida krusei	ATCC 2159	1x
Mycoplasma genitalium	ATCC 33530D	1x
KPC	Zeptometrix NATPPQ-BIO	1x
QNR	ATCC BAA-2728	1x
NDM	Zeptometrix NATPPQ-BIO	1x
mecA	ATCC BAA-2094	1x
mecC	ATCC BAA-2313	1x
VanA	Zeptometrix 0801892	1x
VanB	Zeptometrix 0801953	1x
Sul1	ATCC BAA-3035	1x
Sul2	ATCC BAA-2894	1x
CTX	Zeptometrix NATPPQ-BIO	1x
DfrA	ATCC BAA-3041	1x

10.4. Analytical Specificity (Exclusivity)

10.4.1. In-Slico Analytical Specificity

Primers and probes designed for a target sequence may also bind to similar sequences if they closely match or differ by only a few base pairs from a non-targeted sequence. To ensure specificity to the target sequence, it is essential to screen the primers and probes against the reference database for the intended templates, as well as any databases that may contain potential contaminating templates.

Table 11. 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	*Escherichia coli*	None	None	None
On-Panel	*Streptococcus agalactiae*	None	None	None
On-Panel	*Klebsiella oxytoca*	None	None	None
On-Panel	*Klebsiella michiganensis*	None	None	None
On-Panel	*Staphylococcus saprophyticus*	None	None	None
On-Panel	*Serratia marcescens*	None	None	None
On-Panel	*Proteus mirabilis*	None	None	None
On-Panel	*Mycoplasma hominis*	None	None	None
On-Panel	*Enterococcus spp.*	None	None	None
On-Panel	*Enterobacter cloacae*	None	None	None
On-Panel	*Pseudomonas aeruginosa*	None	None	None
On-Panel	*Citrobacter freundii*	None	None	None
On-Panel	*Citrobacter braakii*	None	None	None
On-Panel	*Klebsiella aerogenes*	None	None	None
On-Panel	*Klebsiella pneumoniae*	None	None	None
On-Panel	*Morganella morganii*	None	None	None
On-Panel	*Prevotella bivia*	None	None	None
On-Panel	*Streptococcus pyogenes*	None	None	None
On-Panel	*Candida dubliniensis*	None	None	None
On-Panel	*Acinetobacter baumannii*	None	None	None
On-Panel	*Staphylococcus epidermidis*	None	None	None
On-Panel	*Staphylococcus aureus*	None	None	None
On-Panel	*Ureaplasma urealyticum*	None	None	None
On-Panel	*Citrobacter koseri*	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	*Bacteroides fragilis*	None	None	None
On-Panel	*Candida tropicalis*	None	None	None
On-Panel	*Candida krusei*	None	None	None
On-Panel	*Mycoplasma genitalium*	None	None	None
On-Panel	KPC	None	None	None

On-Panel	QNR	None	None	None
On-Panel	NDM	None	None	None
On-Panel	mecA	None	None	None
On-Panel	mecC	None	None	None
On-Panel	VanA	None	None	None
On-Panel	VanB	None	None	None
On-Panel	Sul1	None	None	None
On-Panel	Sul2	None	None	None
On-Panel	CTX	None	None	None
On-Panel	DfrA	None	None	None
Off-Panel	*Staphylococcus haemolyticus*	None	None	None
Off-Panel	*Staphylococcus lugdunensis*	None	None	None
Off-Panel	*Streptococcus dysgalactiae*	None	None	None
Off-Panel	*Fusarium solani*	None	None	None
Off-Panel	*Microsporum spp.*	None	None	None
Off-Panel	*Trichophyton spp.*	None	None	None
Off-Panel	*Acinetobacter iwoffi*	None	None	None
Off-Panel	*Acinetobacter nosocomalis*	None	None	None
Off-Panel	*Stenotrophomonas maltophilia*	None	None	None
Off-Panel	*Moraxella catarrhalis*	None	None	None
Off-Panel	*Pasteurella stomatis*	None	None	None
Off-Panel	*Epidermophyton floccosum*	None	None	None
Off-Panel	*Finegoldia magna*	None	None	None
Off-Panel	*Bartonella henselae*	None	None	None
Off-Panel	*Haemophilus influenzae*	None	None	None
Off-Panel	*Candida sojae*	None	None	None
Off-Panel	*Candida oregonensis*	None	None	None
Off-Panel	*Malessezia restricta*	None	None	None
Off-Panel	*Peptoniphilus harei*	None	None	None
Off-Panel	*Peptoniphilus ivorii*	None	None	None
Off-Panel	*Peptostreptococcus prevotii*	None	None	None
Off-Panel	*Peptostreptococcus anaerobius*	None	None	None

Off-Panel	*Listeria monocytogenes*	None	None	None
Off-Panel	*Candida lusitaniae*	None	None	None
Off-Panel	*Kingella kingae*	None	None	None
Off-Panel	*Chlamydia trachomatis*	None	None	None
Off-Panel	*Legionella dumoffii*	None	None	None
Off-Panel	*Corynebacterium diphtheriae*	None	None	None
Off-Panel	*Neisseria meningitidis*	None	None	None
Off-Panel	AmpC	None	None	None
Off-Panel	mcr-3	None	None	None
Off-Panel	SHV	None	None	None
Off-Panel	vanC	None	None	None
Off-Panel	vanD	None	None	None
Off-Panel	SME	None	None	None
Off-Panel	mcr-4	None	None	None
Off-Panel	blaRAHN	None	None	None
Off-Panel	CMY	None	None	None
Off-Panel	ompK36	None	None	None
Off-Panel	SPM	None	None	None
Off-Panel	vanM	None	None	None
Off-Panel	mcr-2	None	None	None
Off-Panel	OXA-24/65	None	None	None
Off-Panel	TEM	None	None	None

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

10.4.2. Wet-Test Analytical Specificity

The potential for non-specific amplification by assays designed to detect analytes was evaluated by testing high concentrations of organisms or nucleic acids using the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit***. On-panel organisms were tested to assess potential intra-panel cross-reactivity, while off-panel organisms were tested to evaluate the specificity of the panel. Off-panel organisms included normal flora, pathogens that may be present in specimens, and genetically related species to those detected by the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit***. The concentration of organisms tested (in triplicate) was at least 1.0E+06 CFU/mL for bacteria, fungi, and parasites, and at least 1.0E+05 units/mL for viruses. For certain organisms that were not available for laboratory testing, in silico analysis of the organism's whole genome sequences was used. The on-panel and off-panel organisms tested are listed in Table 12 and Table 13.

Table 12. On-Panel organisms tested for evaluation of ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Escherichia coli	ATCC 25922	None
Streptococcus agalactiae	ATCC 12386	None
Klebsiella oxytoca	ATCC 700324	None
Klebsiella michiganensis	ATCC 8724	None
Staphylococcus saprophyticus	Zeptometrix 0804014	None
Serratia marcescens	ATCC 29021	None
Proteus mirabilis	Zeptometrix 0801544	None
Mycoplasma hominis	ATCC 27545-TTR	None
Enterococcus spp.	ATCC BAA-2127	None
Enterobacter cloacae	Zeptometrix 0801830	None
Pseudomonas aeruginosa	ATCC 27853	None
Citrobacter freundii	Zeptometrix 0801563	None
Klebsiella aerogenes	ATCC 13048	None
Klebsiella pneumoniae	NCTC 13465	None
Morganella morganii	Zeptometrix 0804010	None
Prevotella bivia	Zeptometrix 0801756	None
Streptococcus pyogenes	Zeptometrix 0801512	None
Candida dubliniensis	Zeptometrix 0801915	None
Acinetobacter baumannii	ATCC 19606	None
Staphylococcus epidermidis	Zeptometrix 0804281	None
Staphylococcus aureus	ATCC 10832	None
Ureaplasma urealyticum	ATCC 27618	None
Citrobacter koseri	Zeptometrix 0801745	None
Citrobacter braakii	Zeptometrix 0804366	None
Candida albicans	ATCC 10231	None
Candida glabrata	ATCC 90030	None
Candida parapsilosis	ATCC 22019	None
Bacteroides fragilis	Zeptometrix 0801583	None
Candida tropicalis	ATCC 750	None

Candida krusei	ATCC 2159	None
Mycoplasma genitalium	ATCC 33530D	None
KPC	Zeptometrix NATPPQ-BIO	None
QNR	ATCC BAA-2728	None
NDM	Zeptometrix NATPPQ-BIO	None
mecA	ATCC BAA-2094	None
mecC	ATCC BAA-2313	None
VanA	Zeptometrix 0801892	None
VanB	Zeptometrix 0801953	None
Sul1	ATCC BAA-3035	None
Sul2	ATCC BAA-2894	None
CTX	Zeptometrix NATPPQ-BIO	None
DfrA	ATCC BAA-3041	None

Table 13. Off-Panel organisms were tested for evaluation of ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Acinetobacter calcoaceticus	ATCC 23055	None
Staphylococcus haemolyticus	Zeptometrix 0801591	None
Staphylococcus lugdunensis	Zeptometrix 0801555	None
Streptococcus dysgalactiae	Zeptometrix 0801516	None
Fusarium solani	Zeptometrix 0801806	None
Acinetobacter iwoffi	Zeptometrix 0801909	None
Stenotrophomonas maltophilia	Zeptometrix 0801569	None
Moraxella catarrhalis	Zeptometrix 0801509	None
Candida sojae	Zeptometrix 0801825	None
Listeria monocytogenes	Zeptometrix 0804339	None
Chlamydia trachomatis	Zeptometrix 0801775	None
Acinetobacter baumannii	ATCC 19606	None
Legionella pneumophilia	Zeptometrix 0801530	None
Neisseria gonorrhoeae	ATCC 19424	None
Neisseria meningitidis	ATCC 13090	None

Aspergillus flavus	Zeptometrix 0801598	None
AmpC	-	None
mcr-3	-	None
SHV	-	None
vanC	-	None
vanD	-	None
SME	-	None
mcr-4	-	None
blaRAHN	-	None
CMY	-	None
ompK36	-	None
SPM	-	None
vanM	-	None
mcr-2	-	None
OXA-24/65	-	None
TEM	-	None

10.5. Interferences

The potential for endogenous or exogenous substances, which may be present in research samples or introduced during sample collection and handling, to interfere with the accurate detection of analytes was evaluated through select direct testing on the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit***. The findings were extrapolated from the interference evaluation of the kit.

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

None of the substances tested were found to interfere with the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit***.

Table 14. Evaluation of potentially interfering substances on the ***MarinaBiolab Pre-Plated Urinary Tract Infection Plus Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Human Genomic DNA	0.07 mg/mL	No Interference
Hemoglobin	10 mg/mL	No Interference
D-Glucose	10 mg/mL	No Interference
Cholesterol	4.0 mg/mL	No Interference
Human Urine	-	No Interference
Competitive Microorganisms		
Escherichia coli	1.0E+06 CFU/mL	No Interference
Streptococcus agalactiae	1.0E+06 CFU/mL	No Interference
Klebsiella oxytoca	1.0E+06 CFU/mL	No Interference
Klebsiella michiganensis	1.0E+06 CFU/mL	No Interference
Staphylococcus saprophyticus	1.0E+06 CFU/mL	No Interference
Serratia marcescens	1.0E+06 CFU/mL	No Interference
Proteus mirabilis	1.0E+06 CFU/mL	No Interference
Mycoplasma hominis	1.0E+06 CFU/mL	No Interference
Enterococcus spp.	1.0E+06 CFU/mL	No Interference
Enterobacter cloacae	1.0E+06 CFU/mL	No Interference
Pseudomonas aeruginosa	1.0E+06 CFU/mL	No Interference
Citrobacter freundii	1.0E+06 CFU/mL	No Interference
Klebsiella aerogenes	1.0E+06 CFU/mL	No Interference
Klebsiella pneumoniae	1.0E+06 CFU/mL	No Interference
Morganella morganii	1.0E+06 CFU/mL	No Interference
Prevotella bivia	1.0E+06 CFU/mL	No Interference
Streptococcus pyogenes	1.0E+06 CFU/mL	No Interference
Candida dubliniensis	1.0E+06 CFU/mL	No Interference
Acinetobacter baumannii	1.0E+06 CFU/mL	No Interference
Staphylococcus epidermidis	1.0E+06 CFU/mL	No Interference
Staphylococcus aureus	1.0E+06 CFU/mL	No Interference
Ureaplasma urealyticum	1.0E+06 CFU/mL	No Interference

Citrobacter koseri	1.0E+06 CFU/mL	No Interference
Citrobacter braakii	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
Bacteroides fragilis	1.0E+06 CFU/mL	No Interference
Candida tropicalis	1.0E+06 CFU/mL	No Interference
Candida krusei	1.0E+06 CFU/mL	No Interference
Mycoplasma genitalium	1.0E+06 CFU/mL	No Interference
KPC	1.0E+06 CFU/mL	No Interference
QNR	1.0E+06 CFU/mL	No Interference
NDM	1.0E+06 CFU/mL	No Interference
mecA	1.0E+06 CFU/mL	No Interference
mecC	1.0E+06 CFU/mL	No Interference
VanA	1.0E+06 CFU/mL	No Interference
VanB	1.0E+06 CFU/mL	No Interference
Sul1	1.0E+06 CFU/mL	No Interference
Sul2	1.0E+06 CFU/mL	No Interference
CTX	1.0E+06 CFU/mL	No Interference
DfrA	1.0E+06 CFU/mL	No Interference
Exogenous Substances		
Feminine Spray/talcum powder	5% v/v	No Interference
Phenazopyridine Hydrochloride (Pyridium)	10 µg/mL	No Interference
Ascorbic acid	0.6 mmol/L	No Interference
High pH	pH = 8.0	No Interference
Low pH	pH = 4.0	No Interference
Antibiotic Pool Amoxicillin trihydrate Metronidazole Tetracycline Hydrochloride Sodium Cefotaxime	1 mg/mL (Each)	No Interference
Specimen Collection Materials		
Urine Tubes (BD Vacutainer® 364992)	N/A	No Interference
Starplex™ Scientific Urine Preservative Tube (22046414)	N/A	No Interference

11. TROUBLESHOOTING

Problem	Cause	Solution
Target-specific and/or internal control (IC) signals were detected in the Negative Control well.	Contamination may arise from the environment, contamination of extraction and/or qPCR reagents, or well-to-well cross-contamination. The signal observed is not true target amplification, but rather background curves generated by the software of the qPCR instrument.	Repeat the qPCR using fresh reagents. Follow the general GLP guidelines in a PCR lab (e.g., decontaminate all surfaces and instruments with sodium hypochlorite or ethanol, and ensure filter tips are used 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 solely for pre-PCR activities. Ignore the Cq value of the No Template Control (NTC) if the amplification curve appears to be background noise rather than a true signal. If the issue persists, contact Technical Support.
No IC signal is detected, but a target-specific signal is observed in the sample wells.	A high copy number of target nucleic acid in the samples leads to 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 for acceptable values specified by the kit, rendering the assay invalid.	The Positive Control was not stored under the recommended conditions. The kit has expired.	Check the kit label for the recommended storage conditions and expiration date. Replace the Positive Control. If necessary, use a new kit.
High Cq values were observed in the repeated samples.	The frozen samples were not mixed properly after thawing. Nucleic acids may be degraded.	Ensure frozen samples are thawed with mild agitation to guarantee thorough mixing. Make sure samples are stored correctly and are not subjected to multiple freeze-thaw cycles.
Target-specific and/or IC signals were detected after 35 cycles in the 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 or IC signals were detected in the sample wells.	Sampling, extraction, or inhibition problem.	Dilute the nucleic acid isolate 1:10 and repeat the qPCR. If the diluted sample does not show a positive result in the IC channel, request a new sample and repeat the nucleic acid extraction. If necessary, repeat the nucleic acid extraction and the qPCR. If the issue persists, request a new sample, repeat the nucleic acid extraction and qPCR. If the problem continues, 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 LLC.

Address: 715 Discovery Blvd, suite 309 Cedar Park, TX 78613

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