RUO

INSTRUCTION FOR USE

UTI+ABR Panel PCR Kit

For Research Use Only

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715 Discovery Blvd, suite 309 Cedar Park, TX 78613

Document Revision History

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Rev.00_April 10, 2025	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 UTI+ABR 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	
KPC	*Streptococcus pyogenes*
VIM	*Escherichia coli*
NDM	*Streptococcus agalactiae*
IMP	*Klebsiella oxytoca*
QNR	*Staphylococcus saprophyticus*
OXA-48-like	*Serratia marcescens*
mecA/C	*Proteus mirabilis*
VanA	*Providencia stuartii*
VanB	*Neisseria gonorrhoeae*
Sul1/2	*Enterobacter cloacae*
CTX	*Pseudomonas aeruginosa*
DfrA	*Klebsiella aerogenes*
ermA	*Klebsiella pneumoniae*
ermB	*Morganella morganii*
ACC	*Enterococcus faecium*
SHV	*Enterococcus faecalis*
DHA	*Acinetobacter baumannii*
TET	*Proteus vulgaris*
TEM	*Staphylococcus aureus*
CMY	*Candida spp*
mefA	*Candida auris*
mphC	*Haemophilus influenzae*
	Mycoplasma hominis
	Peptostreptococcus prevotti
	Chlamydia trachomatis

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 UTI+ABR Panel PCR Kit*** consists of four main components:

1. qPCR Enzyme and Buffer Mix (qPCR Master Mix)
2. Forward, Reverse and Probe Oligo Mix (UTI+ABR Oligo Mix 1-14)
3. A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below (PC-UTI+ABR)
4. DNase/RNase-Free Water (NTC)

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

Table 1. Kit components.

Component	Description	Quantity x Volume
		100 rxn **LEE-UTI 028**
qPCR Master Mix	Ready-to-use mix for qPCR	7 x 1000 µL
UTIP Oligo Mix 1-8	Primers and probes complementary to specific regions of the targets listed in the table above	14 x 250 µL
PC-UTIP	A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below	1 x 400 µL
NTC	DNase/RNase-Free Water	1 x 400 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
UTI+ABR Oligo Mix 1	KPC	**FAM/Green**
	VIM	**HEX/VIC/JOE/Yellow**
	NDM	**ROX/Texas Red/Orange**
	Human RNase P (IC)	**CY5/Red**
UTI+ABR Oligo Mix 2	IMP	**FAM/Green**
	QNR	**HEX/VIC/JOE/Yellow**
	OXA	**ROX/Texas Red/Orange**
	-	**CY5/Red**
UTI+ABR Oligo Mix 3	mecA/C	**FAM/Green**
	VanA	**HEX/VIC/JOE/Yellow**
	VanB	**ROX/Texas Red/Orange**
	-	**CY5/Red**

UTI+ABR Oligo Mix 4	Sul	**FAM/Green**
	CTX	**HEX/VIC/JOE/Yellow**
	DfrA	**ROX/Texas Red/Orange**
	Bacillus atrophaeus (EC)	**CY5/Red**
UTI+ABR Oligo Mix 5	ermA/B	**FAM/Green**
	ACC	**HEX/VIC/JOE/Yellow**
	SHV	**ROX/Texas Red/Orange**
	-	**CY5/Red**
UTI+ABR Oligo Mix 6	*Streptococcus pyogenes*	**FAM/Green**
	-	**HEX/VIC/JOE/Yellow**
	DHA	**ROX/Texas Red/Orange**
	TET	**CY5/Red**
UTI+ABR Oligo Mix 7	*Escherichia coli*	**FAM/Green**
	Streptococcus agalactiae	**HEX/VIC/JOE/Yellow**
	Klebsiella oxytoca	**ROX/Texas Red/Orange**
	TEM	**CY5/Red**
UTI+ABR Oligo Mix 8	*Staphylococcus saprophyticus*	**FAM/Green**
	Serratia marcescens	**HEX/VIC/JOE/Yellow**
	Proteus mirabilis	**ROX/Texas Red/Orange**
	Providencia stuartii	**CY5/Red**
UTI+ABR Oligo Mix 9	*Neisseria gonorrhoeae*	**FAM/Green**
	Enterobacter cloacae	**HEX/VIC/JOE/Yellow**
	Pseudomonas aeruginosa	**ROX/Texas Red/Orange**
	-	**CY5/Red**
UTI+ABR Oligo Mix 10	*Klebsiella aerogenes*	**FAM/Green**
	Klebsiella pneumoniae	**HEX/VIC/JOE/Yellow**
	Morganella morganii	**ROX/Texas Red/Orange**
	-	**CY5/Red**
UTI+ABR Oligo Mix 11	*Enterococcus faecium*	**FAM/Green**
	Enterococcus faecalis	**HEX/VIC/JOE/Yellow**
	Acinetobacter baumannii	**ROX/Texas Red/Orange**
	Proteus vulgaris	**CY5/Red**

UTI+ABR Oligo Mix 12	*Staphylococcus aureus*	**FAM/Green**
	Candida spp.	**HEX/VIC/JOE/Yellow**
	Chlamydia trachomatis	**ROX/Texas Red/Orange**
	-	**CY5/Red**
UTI+ABR Oligo Mix 13	*Haemophilus influenzae*	**FAM/Green**
	-	**HEX/VIC/JOE/Yellow**
	Mycoplasma hominis	**ROX/Texas Red/Orange**
	CMY	**CY5/Red**
UTI+ABR Oligo Mix 14	*Peptostreptococcus prevotti*	**FAM/Green**
	mefA	**HEX/VIC/JOE/Yellow**
	mphC	**ROX/Texas Red/Orange**
	Candida auris	**CY5/Red**

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/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 UTI+ABR 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 UTI+ABR Panel PCR Kit*** is shipped on dry ice. If any component, except the qPCR Master Mix, 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

- Completely thaw all components at room temperature for at least 30 minutes prior to use.
- Once thawed, keep all components on ice throughout the entire testing procedure.
- 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)
        - No Template Addition Control (NRC)
- Vortex and briefly centrifuge all components before each use.
- Prepare a master mix by combining the required components for the total number of reactions plus an additional 10% to account for pipetting variability.

Table 3. Reaction set-up.

Reaction Mix Component	1X Reaction (µL) per well
qPCR Master Mix	5 µL
UTI+ABR Oligo Mix 1-14	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 UTI+ABR Oligo Mix 1-14 to each PCR tube.
- Add 2.5 µL of the isolated sample to the corresponding tubes.
- The final reaction volume should be 10 µL.
- Close the tubes, centrifuge briefly, then place them into the real-time PCR instrument.
- Proceed with amplification using the PCR profile outlined below.

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 UTI+ABR 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 5. Instrument-specific settings.

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 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 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 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 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	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 8. 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 UTI+ABR 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 UTI+ABR Panel PCR Kit*** reportable targets, are presented in Table 9 below.

Table 9. Summary of LoD study results.

Analyte	Isolate ID/Source	LoD Concentration (copies/mL)	Detected/Total
KPC	Zeptometrix NATPPQ-BIO	1.4E+02 copies/mL	**20/20** 100%
VIM	Zeptometrix NATPPQ-BIO	7.8E+01 copies/mL	**20/20** 100%
NDM	Zeptometrix NATPPQ-BIO	8.5E+01 copies/mL	**20/20** 100%
IMP	Zeptometrix NATPPQ-BIO	9.7E+01 copies/mL	**20/20** 100%
QNR	ATCC BAA-2728	1.1E+02 copies/mL	**20/20** 100%
OXA-48-like	Zeptometrix NATPPQ-BIO	1.5E+02 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%
ermA	ATCC BAA-1415	1.2E+02 copies/mL	**20/20** 100%
ermB	ATCC BAA-1415	1.8E+02 copies/mL	**20/20** 100%
ACC	In-house	8.3E+01 copies/mL	**20/20** 100%
SHV	ATCC BAA-3261	1.1E+02 copies/mL	**20/20** 100%
DHA	In-house	2.2E+02 copies/mL	**20/20** 100%
TET	ATCC BAA-3288	1.4E+02 copies/mL	**20/20** 100%
TEM	ATCC BAA-3263	6.8E+01 copies/mL	**20/20** 100%
CMY	ATCC BAA-3286	7.9E+01 copies/mL	**20/20** 100%
mefA	In-house	1.3E+02 copies/mL	**20/20** 100%
mphC	In-house	1.4E+02 copies/mL	**20/20** 100%
Streptococcus pyogenes	Zeptometrix 0801512	3.5E+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%
Klebsiella oxytoca	ATCC 700324	2.6E+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%

Providencia stuartii	Zeptometrix 0810452CF	4.5E+01 copies/mL	**20/20** 100%
Neisseria gonorrhoeae	ATCC 19424	6.0E+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%
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%
Enterococcus faecium	ATCC BAA-2127	4.5E+01 copies/mL	**20/20** 100%
Enterococcus faecalis	Zeptometrix 0804216	3.6E+02 copies/mL	**20/20** 100%
Acinetobacter baumannii	ATCC 19606	1.7E+02 copies/mL	**20/20** 100%
Proteus vulgaris	Zeptometrix 0810290CF	1.5E+02 copies/mL	**20/20** 100%
Staphylococcus aureus	ATCC 10832	5.5E+01 copies/mL	**20/20** 100%
Chlamydia trachomatis	Zeptometrix 0801775	5.8E+01 copies/mL	**20/20** 100%
Haemophilus influenzae	ATCC 33391	7.0E+01 copies/mL	**20/20** 100%
Mycoplasma hominis	ATCC 27545-TTR	1.1E+02 copies/mL	**20/20** 100%
Candida auris	ATCC MYA-5003	7.2E+01 copies/mL	**19/20** 95%
Candida spp.	ATCC 10231	3.4E+02 copies/mL	**20/20** 100%
Peptostreptococcus prevotti	ATCC 9321	2.4E+02 copies/mL	**19/20** 95%

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 KPC, VIM, NDM, IMP, QNR, OXA-48-like, mecA, mecC, VanA, VanB, Sul1, Sul2, CTX, DfrA, ermA, ermB, ACC, SHV, DHA, TET, TEM, CMY, mefA, mphC, *Streptococcus pyogenes, Escherichia coli, Streptococcus agalactiae, Klebsiella oxytoca, Staphylococcus saprophyticus, Serratia marcescens, Proteus mirabilis, Providencia stuartii, Neisseria gonorrhoeae, Enterobacter cloacae, Pseudomonas aeruginosa, Klebsiella aerogenes, Klebsiella pneumoniae, Morganella morganii, Enterococcus faecium, Enterococcus faecalis, Acinetobacter baumannii, Proteus vulgaris, Staphylococcus aureus, Chlamydia trachomatis, Haemophilus influenzae, Mycoplasma hominis, Candida auris, Candida spp.,* and *Peptostreptococcus prevotti* 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 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
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%
VIM	Sense Primer	1198	99.12%	0.88%	0.00%	0.00%
VIM	Antisense Primer	1198	99.12%	0.88%	0.00%	0.00%
VIM	Hydrolysis Probe	1125	99.05%	0.95%	0.00%	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%
IMP	Sense Primer	973	99.44%	0.56%	0.00%	0.00%
IMP	Antisense Primer	973	99.44%	0.56%	0.00%	0.00%
IMP	Hydrolysis Probe	958	99.24%	0.76%	0.00%	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%
OXA-48-like	Sense Primer	538	99.40%	0.60%	0.00%	0.00%
OXA-48-like	Antisense Primer	538	99.40%	0.60%	0.00%	0.00%
OXA-48-like	Hydrolysis Probe	520	99.20%	0.80%	0.00%	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%
CTX	Sense Primer	4844	99.88%	0.12%	0.00%	0.00%
CTX	Antisense Primer	4844	99.88%	0.12%	0.00%	0.00%
CTX	Hydrolysis Probe	4810	99.80%	0.20%	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%
ermA	Sense Primer	1.982	100.00%	0.00%	0.00%	0.00%
ermA	Antisense Primer	1.982	100.00%	0.00%	0.00%	0.00%
ermA	Hydrolysis Probe	1.987	100.00%	0.00%	0.00%	0.00%
ermB	Sense Primer	2.899	100.00%	0.00%	0.00%	0.00%
ermB	Antisense Primer	2.899	99.14%	0.86%	0.00%	0.00%

ermB	Hydrolysis Probe	2.890	100.00%	0.00%	0.00%	0.00%
ACC	Sense Primer	65	100.00%	0.00%	0.00%	0.00%
ACC	Antisense Primer	65	100.00%	0.00%	0.00%	0.00%
ACC	Hydrolysis Probe	71	100.00%	0.00%	0.00%	0.00%
SHV	Sense Primer	5.463	100.00%	0.00%	0.00%	0.00%
SHV	Antisense Primer	5.463	100.00%	0.00%	0.00%	0.00%
SHV	Hydrolysis Probe	5.470	100.00%	0.00%	0.00%	0.00%
DHA	Sense Primer	798	100.00%	0.00%	0.00%	0.00%
DHA	Antisense Primer	798	99.82%	0.18%	0.00%	0.00%
DHA	Hydrolysis Probe	792	100.00%	0.00%	0.00%	0.00%
TET	Sense Primer	49.900	100.00%	0.00%	0.00%	0.00%
TET	Antisense Primer	49.900	97.45%	2.55%	0.00%	0.00%
TET	Hydrolysis Probe	49.915	99.26%	0.74%	0.00%	0.00%
TEM	Sense Primer	462	100.00%	0.00%	0.00%	0.00%
TEM	Antisense Primer	462	98.50%	1.50%	0.00%	0.00%
TEM	Hydrolysis Probe	465	99.00%	1.00%	0.00%	0.00%
CMY	Sense Primer	1.090	100.00%	0.00%	0.00%	0.00%
CMY	Antisense Primer	1.090	100.00%	0.00%	0.00%	0.00%
CMY	Hydrolysis Probe	1.091	100.00%	0.00%	0.00%	0.00%
mefA	Sense Primer	63	100.00%	0.00%	0.00%	0.00%
mefA	Antisense Primer	63	100.00%	0.00%	0.00%	0.00%
mefA	Hydrolysis Probe	65	100.00%	0.00%	0.00%	0.00%
mphC	Sense Primer	209	100.00%	0.00%	0.00%	0.00%
mphC	Antisense Primer	209	100.00%	0.00%	0.00%	0.00%
mphC	Hydrolysis Probe	215	100.00%	0.00%	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%
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%
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%
Providencia stuartii	Sense Primer	23	100.00%	0.00%	0.00%	0.00%
Providencia stuartii	Antisense Primer	23	100.00%	0.00%	0.00%	0.00%
Providencia stuartii	Hydrolysis Probe	22	100.00%	0.00%	0.00%	0.00%
Neisseria gonorrhoeae	Sense Primer	597	99.20%	0.80%	0.00%	0.00%
Neisseria gonorrhoeae	Antisense Primer	597	99.20%	0.80%	0.00%	0.00%
Neisseria gonorrhoeae	Hydrolysis Probe	590	99.05%	0.80%	0.05%	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%
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%
Enterococcus faecium	Sense Primer	552	98.68%	1.32%	0.00%	0.00%
Enterococcus faecium	Antisense Primer	555	98.68%	1.32%	0.00%	0.00%
Enterococcus faecium	Hydrolysis Probe	555	98.46%	1.54%	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%
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%
Proteus vulgaris	Sense Primer	155	99.83%	0.17%	0.00%	0.00%
Proteus vulgaris	Antisense Primer	155	99.83%	0.17%	0.00%	0.00%
Proteus vulgaris	Hydrolysis Probe	157	99.85%	0.15%	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%
Chlamydia trachomatis	Sense Primer	862	99.64%	0.36%	0.00%	0.00%
Chlamydia trachomatis	Antisense Primer	862	99.64%	0.36%	0.00%	0.00%
Chlamydia trachomatis	Hydrolysis Probe	846	99.60%	0.40%	0.00%	0.00%
Haemophilus influenzae	Sense Primer	153	98.25%	1.75%	0.00%	0.00%
Haemophilus influenzae	Antisense Primer	153	98.68%	1.32%	0.00%	0.00%
Haemophilus influenzae	Hydrolysis Probe	121	99.93%	0.07%	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%
Candida auris	Sense Primer	501	100,00%	0,00%	0.00%	0.00%
Candida auris	Antisense Primer	501	100,00%	0,00%	0.00%	0.00%
Candida auris	Hydrolysis Probe	499	100,00%	0,00%	0.00%	0.00%
Candida spp	Sense Primer	4242	98.21%	1.79%	0.00%	0.00%
Candida spp	Antisense Primer	4242	98.21%	1.79%	0.00%	0.00%

Candida spp	Hydrolysis Probe	4143	98.02%	1.98%	0.00%	0.00%
Peptostreptococcus prevotti	Sense Primer	88	99.44%	0.56%	0.00%	0.00%
Peptostreptococcus prevotti	Antisense Primer	88	99.44%	0.56%	0.00%	0.00%
Peptostreptococcus prevotti	Hydrolysis Probe	92	99.24%	0.76%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab UTI+ABR 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 UTI+ABR 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 11 below.

Table 11. Results of the wet inclusivity test.

Variant/Type/Subtype/Lineage/Genotype/Species	Isolate ID/Source	xLoD Detected
KPC	Zeptometrix NATPPQ-BIO	1x
VIM	Zeptometrix NATPPQ-BIO	1x
NDM	Zeptometrix NATPPQ-BIO	1x
IMP	Zeptometrix NATPPQ-BIO	1x
QNR	ATCC BAA-2728	1x
OXA-48-like	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
ermA	ATCC BAA-1415	1x
ermB	ATCC BAA-1415	1x
ACC	In-house	1x

SHV	ATCC BAA-3261	1x
DHA	In-house	1x
TET	ATCC BAA-3288	1x
TEM	ATCC BAA-3263	1x
CMY	ATCC BAA-3286	1x
mefA	In-house	1x
mphC	In-house	1x
Streptococcus pyogenes	Zeptometrix 0801512	1x
Escherichia coli	ATCC 25922	1x
Streptococcus agalactiae	ATCC 12386	1x
Klebsiella oxytoca	ATCC 700324	1x
Staphylococcus saprophyticus	Zeptometrix 0804014	1x
Serratia marcescens	ATCC 29021	1x
Proteus mirabilis	Zeptometrix 0801544	1x
Providencia stuartii	Zeptometrix 0810452CF	1x
Neisseria gonorrhoeae	ATCC 19424	1x
Enterobacter cloacae	Zeptometrix 0801830	1x
Pseudomonas aeruginosa	ATCC 27853	1x
Klebsiella aerogenes	ATCC 13048	1x
Klebsiella pneumoniae	NCTC 13465	1x
Morganella morganii	Zeptometrix 0804010	1x
Enterococcus faecium	ATCC BAA-2127	1x
Enterococcus faecalis	Zeptometrix 0804216	1x
Acinetobacter baumannii	ATCC 19606	1x
Proteus vulgaris	Zeptometrix 0810290CF	1x
Staphylococcus aureus	ATCC 10832	1x
Chlamydia trachomatis	Zeptometrix 0801775	1x
Haemophilus influenzae	ATCC 33391	1x
Mycoplasma hominis	ATCC 27545-TTR	1x
Candida auris	ATCC MYA-5003	1x
Candida spp.	ATCC 10231	1x
Peptostreptococcus prevotti	ATCC 9321	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 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	KPC	None	None	None
On-Panel	VIM	None	None	None
On-Panel	NDM	None	None	None
On-Panel	IMP	None	None	None
On-Panel	QNR	None	None	None
On-Panel	OXA-48-like	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
On-Panel	ermA	None	None	None
On-Panel	ermB	None	None	None
On-Panel	ACC	None	None	None
On-Panel	SHV	None	None	None
On-Panel	DHA	None	None	None
On-Panel	TET	None	None	None
On-Panel	TEM	None	None	None
On-Panel	CMY	None	None	None
On-Panel	mefA	None	None	None

On-Panel	mphC	None	None	None
On-Panel	*Streptococcus pyogenes*	None	None	None
On-Panel	*Escherichia coli*	None	None	None
On-Panel	*Streptococcus agalactiae*	None	None	None
On-Panel	*Klebsiella oxytoca*	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	*Providencia stuartii*	None	None	None
On-Panel	*Neisseria gonorrhoeae*	None	None	None
On-Panel	*Enterobacter cloacae*	None	None	None
On-Panel	*Pseudomonas aeruginosa*	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	*Enterococcus faecium*	None	None	None
On-Panel	*Enterococcus faecalis*	None	None	None
On-Panel	*Acinetobacter baumannii*	None	None	None
On-Panel	*Proteus vulgaris*	None	None	None
On-Panel	*Staphylococcus aureus*	None	None	None
On-Panel	*Chlamydia trachomatis*	None	None	None
On-Panel	*Haemophilus influenzae*	None	None	None
On-Panel	*Mycoplasma hominis*	None	None	None
On-Panel	*Candida auris*	None	None	None
On-Panel	*Candida spp.*	None	None	None
On-Panel	*Peptostreptococcus prevotti*	None	None	None
Off-Panel	*Staphylococcus epidermidis*	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	*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

* 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 UTI+ABR 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 UTI+ABR 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 13 and Table 14.

Table 13. On-Panel organisms tested for evaluation of ***MarinaBiolab UTI+ABR Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
KPC	Zeptometrix NATPPQ-BIO	None
VIM	Zeptometrix NATPPQ-BIO	None
NDM	Zeptometrix NATPPQ-BIO	None
IMP	Zeptometrix NATPPQ-BIO	None
QNR	ATCC BAA-2728	None
OXA-48-like	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
ermA	ATCC BAA-1415	None
ermB	ATCC BAA-1415	None
ACC	In-house	None
SHV	ATCC BAA-3261	None
DHA	In-house	None
TET	ATCC BAA-3288	None
TEM	ATCC BAA-3263	None
CMY	ATCC BAA-3286	None
mefA	In-house	None
mphC	In-house	None
Streptococcus pyogenes	Zeptometrix 0801512	None
Escherichia coli	ATCC 25922	None
Streptococcus agalactiae	ATCC 12386	None
Klebsiella oxytoca	ATCC 700324	None
Staphylococcus saprophyticus	Zeptometrix 0804014	None
Serratia marcescens	ATCC 29021	None

Proteus mirabilis	Zeptometrix 0801544	None
Providencia stuartii	Zeptometrix 0810452CF	None
Neisseria gonorrhoeae	ATCC 19424	None
Enterobacter cloacae	Zeptometrix 0801830	None
Pseudomonas aeruginosa	ATCC 27853	None
Klebsiella aerogenes	ATCC 13048	None
Klebsiella pneumoniae	NCTC 13465	None
Morganella morganii	Zeptometrix 0804010	None
Enterococcus faecium	ATCC BAA-2127	None
Enterococcus faecalis	Zeptometrix 0804216	None
Acinetobacter baumannii	ATCC 19606	None
Proteus vulgaris	Zeptometrix 0810290CF	None
Staphylococcus aureus	ATCC 10832	None
Chlamydia trachomatis	Zeptometrix 0801775	None
Haemophilus influenzae	ATCC 33391	None
Mycoplasma hominis	ATCC 27545-TTR	None
Candida auris	ATCC MYA-5003	None
Candida spp.	ATCC 10231	None
Peptostreptococcus prevotti	ATCC 9321	None

Table 14. Off-Panel organisms were tested for evaluation of ***MarinaBiolab UTI+ABR Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Acinetobacter calcoaceticus	ATCC 23055	None
Staphylococcus epidermidis	Zeptometrix 0804281	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
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

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 UTI+ABR 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 UTI+ABR Panel PCR Kit***.

Table 15. Evaluation of potentially interfering substances on the ***MarinaBiolab UTI+ABR Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Whole Blood	10% v/v	No Interference
Human serum	5% v/v	No Interference
Human Urine	-	No Interference
Competitive Microorganisms		
KPC	1.0E+06 CFU/mL	No Interference
VIM	1.0E+06 CFU/mL	No Interference
NDM	1.0E+06 CFU/mL	No Interference
IMP	1.0E+06 CFU/mL	No Interference
QNR	1.0E+06 CFU/mL	No Interference
OXA-48-like	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
ermA	1.0E+06 CFU/mL	No Interference
ermB	1.0E+06 CFU/mL	No Interference
ACC	1.0E+06 CFU/mL	No Interference
SHV	1.0E+06 CFU/mL	No Interference
DHA	1.0E+06 CFU/mL	No Interference
TET	1.0E+06 CFU/mL	No Interference
TEM	1.0E+06 CFU/mL	No Interference
CMY	1.0E+06 CFU/mL	No Interference
mefA	1.0E+06 CFU/mL	No Interference
mphC	1.0E+06 CFU/mL	No Interference
Streptococcus pyogenes	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
Klebsiella oxytoca	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
Providencia stuartii	1.0E+06 CFU/mL	No Interference
Neisseria gonorrhoeae	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
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

Enterococcus faecium	1.0E+06 CFU/mL	No Interference
Enterococcus faecalis	1.0E+06 CFU/mL	No Interference
Acinetobacter baumannii	1.0E+06 CFU/mL	No Interference
Proteus vulgaris	1.0E+06 CFU/mL	No Interference
Staphylococcus aureus	1.0E+06 CFU/mL	No Interference
Chlamydia trachomatis	1.0E+06 CFU/mL	No Interference
Haemophilus influenzae	1.0E+06 CFU/mL	No Interference
Mycoplasma hominis		
Candida auris	5% v/v	No Interference
Candida spp.	10 µg/mL	No Interference
Peptostreptococcus prevotti	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
RUO	Research Use Only		Use-by date
	Manufacturer	LOT	Batch code
CONTROL –	Negative control	NON STERILE	Non-sterile
CONTROL +	Positive control		Consult instructions for use or consult electronic instructions for use
CONTROL	Control		Caution
	Temperature limit	REF	Catalogue number
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Keep dry		Keep upright
Σ	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.