RUO

INSTRUCTION FOR USE

Pre-Plated Respiratory Pathogen Panel PCR Kit

For Research Use Only

Σ 96

REF PP-RPP 002

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Document Revision History

Rev.No_Date	Revision Description
Rev.00_June 20, 2024	First Release
Rev.01_April 10, 2025	Minor Revision
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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 Respiratory Pathogen Panel PCR Kit*** is a multiplex, qualitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-qPCR) test intended for the simultaneous detection and identification of multiple pathogenic nucleic acids in research samples. The kit enables RT-qPCR results in less than one hour. It is designed to detect gene sequences from the following organisms:

Targets	
SARS-CoV-2	*Streptococcus pyogenes*
Influenza A	*Moraxella catarrhalis*
Influenza B	*Staphylococcus aureus*
Coronavirus 229E	*Klebsiella pneumoniae*
Coronavirus OC43	*Legionella pneumophila*
Coronavirus NL63	*Chlamydia pneumoniae*
Coronavirus HKU1	*Mycoplasma pneumoniae*
Respiratory Syncytial Virus A	*Haemophilus influenzae*
Respiratory Syncytial Virus B	*Streptococcus pneumoniae*
Human Rhinovirus	*Bordetella pertussis*
Human Bocavirus	*Enterobacter cloacae*
Human Metapneumovirus	*Acinetobacter baumannii*
Parainfluenza Virus 1	*Klebsiella aerogenes*
Parainfluenza Virus 2	*Proteus mirabilis*
Parainfluenza Virus 3	*Pseudomonas aeruginosa*
Parainfluenza Virus 4	*Staphylococcus epidermidis*
Enterovirus	mecA/C
Adenovirus	
Epstein–Barr Virus	
Controls	
Human RNase P (IC)	
Bacillus atrophaeus (EC)	
MS2 Bacteriophage (EC)	

2. PRINCIPLE of the PROCEDURE

From the RNA and DNA target regions in lysed or extracted research samples, the RNA is first reverse transcribed into complementary DNA (cDNA) using reverse transcriptase. Both cDNA and DNA target regions are then 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 RNA or 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 Respiratory Pathogen Panel PCR Kit*** consists of three main components:

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

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

Table 1. Kit components.

Component	Description	Quantity x Volume
		96 rxn PP-RPP 002
Pre-Plated RPP Plus Mix 1-8 Strip 1	Ready-to-use mix for RT-qPCR	96 Strips (15 µL)
Pre-Plated RPP Plus Mix 9-11 Strip 2	Ready-to-use mix for RT-qPCR	96 Strips (15 µL)
PC-RPP Plus	A mixture of non-infectious cDNA and DNA from artificial samples, including the targets listed in the table below	2 x 400 µL
NTC-RPP Plus	DNase/RNase-Free Water	2 x 400 µL
PC-RPP Plus Pre-Mix	A mixture of non-infectious cDNA and DNA from artificial samples + Oligo + Master Mix	1 x 200 µL
NTC-RPP 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
RPP Plus Oligo Mix 1	SARS-CoV-2	FAM
	Influenza A	HEX/VIC/JOE
	Influenza B	ROX/Texas Red
	Human RNase P (IC)	CY5
RPP Plus Oligo Mix 2	Coronavirus 229E	FAM
	Coronavirus OC43	HEX/VIC/JOE
	Coronavirus NL63	ROX/Texas Red
	Respiratory Syncytial Virus A	CY5
	Streptococcus pyogenes	FAM
	Moraxella catarrhalis	HEX/VIC/JOE

RPP Plus Oligo Mix 3	*Staphylococcus aureus*	ROX/Texas Red
	Respiratory Syncytial Virus B	CY5

RPP Plus Oligo Mix 4	Human Rhinovirus	FAM
	Human Bocavirus	HEX/VIC/JOE
	Human Metapneumovirus	ROX/Texas Red
	Parainfluenza Virus 1	CY5
RPP Plus Oligo Mix 5	Parainfluenza Virus 2	FAM
	Enterovirus	HEX/VIC/JOE
	Coronavirus HKU1	ROX/Texas Red
	Parainfluenza Virus 3	CY5
RPP Plus Oligo Mix 6	Adenovirus	FAM
	Klebsiella pneumoniae	HEX/VIC/JOE
	Epstein-Barr Virus	ROX/Texas Red
	Parainfluenza Virus 4	CY5
RPP Plus Oligo Mix 7	*Legionella pneumophila*	FAM
	Chlamydia pneumoniae	HEX/VIC/JOE
	Mycoplasma pneumoniae	ROX/Texas Red
	MS2 Bacteriophage (EC)	CY5
RPP Plus Oligo Mix 8	*Haemophilus influenzae*	FAM
	Streptococcus pneumoniae	HEX/VIC/JOE
	Bordetella pertussis	ROX/Texas Red
	Bacillus atrophaeus (EC)	CY5
RPP Plus Oligo Mix 9	mecA/C	FAM
	Enterobacter cloacae	HEX/VIC/JOE
	Acinetobacter baumannii	ROX/Texas Red
	-	CY5
RPP Plus Oligo Mix 10	*Klebsiella aerogenes*	FAM
	-	HEX/VIC/JOE
	Proteus mirabilis	ROX/Texas Red
	-	CY5
RPP Plus Oligo Mix 111	-	FAM
	-	HEX/VIC/JOE
	Pseudomonas aeruginosa	ROX/Texas Red
	Staphylococcus epidermidis	CY5

The oligonucleotide set targeting the human *RNase P* mRNA (Internal Control: IC), *Bacillus atrophaeus* (External Control: EC) and MS2 Bacteriophage (EC) are used to monitor sampling, nucleic acid extraction, reverse transcription, and inhibition of both reverse transcription and qPCR. The kit also contains negative and positive control templates to evaluate contamination and the RT-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 Respiratory Pathogen 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 Respiratory Pathogen 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

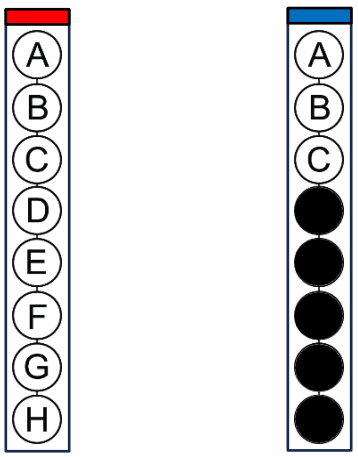
Samples intended for nucleic acid isolation must be collected using appropriate cell collection systems. The performance of the kit is highly dependent on both the quantity and quality of the extracted nucleic acid. Ensure that the extraction method used is compatible with real-time PCR technology.

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.

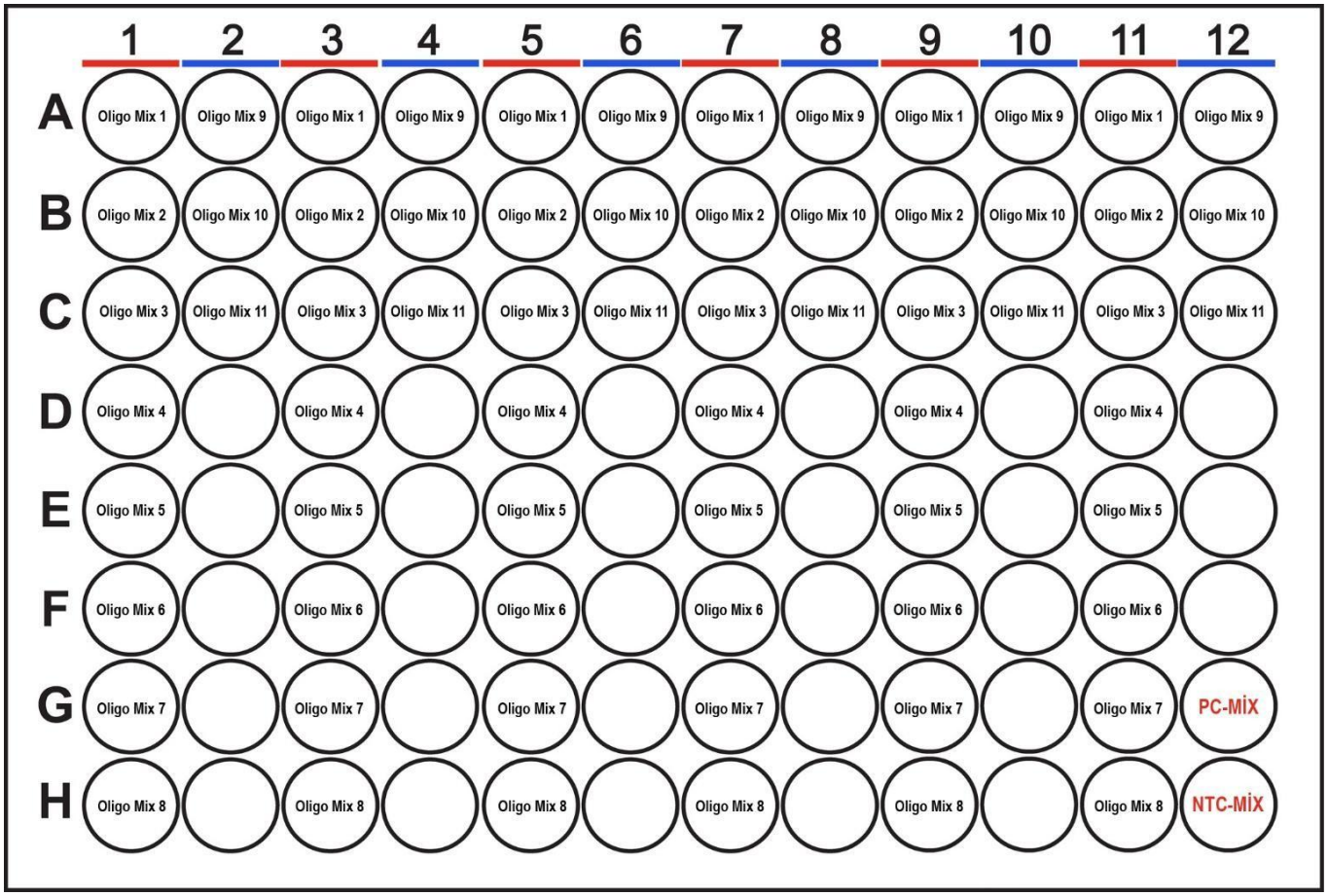


Table 3. Amplification profile.

Step	Number of Cycles	Temperature	Time	Data Collection
Reverse Transcription	1	52 °C	5 min	FAM HEX/VIC/JOE ROX/Texas Red CY5
Initial Denaturation	1	95 °C	10 sec	
Denaturation	40	95 °C	5 sec	
Annealing/Extension		55 °C	15 sec	

8. INTERPRETATION OF RESULTS

MarinaBiolab Pre-Plated Respiratory Pathogen 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	RT-qPCR reaction setup and reagent integrity	+	+
Internal/External Control	To monitor the integrity of nucleic acid extraction and RT-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 RNA pathogens.

RNA Pathogens	Internal Control (*RNase P*)	External Control (*MS2*)	Results	Interpretation
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive for Target	Target RNA is detected
Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Positive for Target	Target RNA is detected
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive for Target	Target RNA 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 RNA is not detected
Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative for Target	Target RNA is not detected
Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Negative for Target	Target RNA 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.

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 Pre-Plated Respiratory Pathogen 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 Respiratory Pathogen 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
SARS-CoV-2	ATCC VR-1986HK	1.2E+02 copies/mL	**20/20** 100%
Influenza A	Zeptometrix 0810036CF	1.1E+02 copies/mL	**20/20** 100%
Influenza B	Zeptometrix 0810255CF	8.7E+01 copies/mL	**20/20** 100%
Coronavirus 229E	Zeptometrix 0810229CF	6.5E+01 copies/mL	**20/20** 100%
Coronavirus OC43	Zeptometrix 0810024CF	9.7E+01 copies/mL	**20/20** 100%
Coronavirus NL63	Zeptometrix 0810228CF	8.4E+01 copies/mL	**19/20** 95%
Coronavirus HKU1	ATCC VR-3262SD	1.1E+02 copies/mL	**20/20** 100%
Parainfluenza Virus 1	Zeptometrix 0810014CF	1.6E+02 copies/mL	**20/20** 100%
Parainfluenza Virus 2	Zeptometrix 0810015CF	1.4E+02 copies/mL	**20/20** 100%
Parainfluenza Virus 3	Zeptometrix 0810016CF	9.6E+01 copies/mL	**20/20** 100%
Parainfluenza Virus 4	Zeptometrix 0810060CF	8.7E+01 copies/mL	**20/20** 100%
Human Rhinovirus	Zeptometrix 0810012CFN	1.3E+02 copies/mL	**20/20** 100%

Enterovirus	Zeptometrix 0810300CF	1.5E+02 copies/mL	**20/20** 100%
Adenovirus	Zeptometrix 0810062CF	1.1E+02 copies/mL	**20/20** 100%
Human Bocavirus	ATCC VR-3251SD	7.7E+01 copies/mL	**19/20** 95%
Respiratory Syncytial Virus A	Zeptometrix 0810040ACF	9.9E+01 copies/mL	**20/20** 100%
Respiratory Syncytial Virus B	Zeptometrix 0810040CF	1.3E+02 copies/mL	**20/20** 100%
Human Metapneumovirus	Zeptometrix 0810161CF	7.6E+01 copies/mL	**20/20** 100%
Epstein–Barr Virus	Zeptometrix 0810008CF	6.7E+01 copies/mL	**20/20** 100%
Streptococcus pyogenes	Zeptometrix 0801512	3.5E+01 copies/mL	**20/20** 100%
Legionella pneumophila	Zeptometrix 0801645	6.8E+01 copies/mL	**20/20** 100%
Mycoplasma pneumoniae	Zeptometrix 0801579	8.8E+01 copies/mL	**20/20** 100%
Chlamydia pneumoniae	Zeptometrix 0804392	8.0E+01 copies/mL	**19/20** 95%
Haemophilus influenzae	ATCC 33391	7.0E+01 copies/mL	**20/20** 100%
Bordetella pertussis	ATCC 9797	1.0E+02 copies/mL	**20/20** 100%
Streptococcus pneumoniae	ATCC 33400	1.1E+02 copies/mL	**20/20** 100%
Moraxella catarrhalis	ATCC 25238	7.3E+01 copies/mL	**20/20** 100%
Staphylococcus aureus	ATCC 12600	8.6E+01 copies/mL	**20/20** 100%
Klebsiella pneumoniae	ATCC 13883	9.2E+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%

Enterobacter cloacae	Zeptometrix 0801830	7.4E+01 copies/mL	**19/20** 95%
Acinetobacter baumannii	ATCC 19606	1.7E+02 copies/mL	**20/20** 100%
Klebsiella aerogenes	ATCC 13048	2.4E+02 copies/mL	**20/20** 100%
Proteus mirabilis	Zeptometrix 0801544	2.1E+02 copies/mL	**20/20** 100%
Pseudomonas aeruginosa	ATCC 27853	6.7E+02 copies/mL	**20/20** 100%
Staphylococcus epidermidis	Zeptometrix 0804281	4.9E+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 SARS-CoV-2, Influenza A, Influenza B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus, Enterovirus, Adenovirus, Human Bocavirus, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Human Metapneumovirus, Epstein–Barr Virus, *Streptococcus pyogenes, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, Haemophilus influenzae, Bordetella pertussis, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Klebsiella pneumoniae*, *Enterobacter cloacae, Acinetobacter baumannii, Klebsiella aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus epidermidis,* mecA and mecC 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
SARS-CoV-2	ORF1ab Sense Primer	54254	96.60%	3.40%	0.00%	0.00%
SARS-CoV-2	ORF1ab Antisense Primer	54254	96.42%	3.56%	0.02%	0.00%
SARS-CoV-2	ORF1ab Hydrolysis Probe	55425	95.62%	4.38%	0.00%	0.00%
SARS-CoV-2	N Sense Primer	54626	96.42%	3.58%	0.00%	0.00%
SARS-CoV-2	N Antisense Primer	54626	97.27%	2.63%	0.00%	0.00%
SARS-CoV-2	N Hydrolysis Probe	54988	95.45%	4.55%	0.00%	0.00%
Influenza A	Sense Primer	47.865	92.24%	7.65%	0.11%	0.00%
Influenza A	Antisense Primer	47.865	97.52%	2.48%	0.00%	0.00%
Influenza A	Hydrolysis Probe	49.224	96.56%	3.44%	0.00%	0.00%
Influenza B	Sense Primer	7.945	98.89%	1.11%	0.00%	0.00%
Influenza B	Antisense Primer	7.945	99.87%	0.13%	0.00%	0.00%
Influenza B	Hydrolysis Probe	7.947	99.57%	0.43%	0.00%	0.00%
Coronavirus 229E	Sense Primer	279	99.99%	0.005%	0.00%	0.00%
Coronavirus 229E	Antisense Primer	279	99.99%	0.005%	0.00%	0.00%
Coronavirus 229E	Hydrolysis Probe	280	99.94%	0.06%	0.00%	0.00%
Coronavirus OC43	Sense Primer	986	97.45%	2.55%	0.00%	0.00%
Coronavirus OC43	Antisense Primer	986	98.52%	1.48%	0.00%	0.00%
Coronavirus OC43	Hydrolysis Probe	1049	95.88%	4.12%	0.00%	0.00%
Coronavirus NL63	Sense Primer	242	98.75%	1.25%	0.00%	0.00%
Coronavirus NL63	Antisense Primer	242	98.75%	1.25%	0.00%	0.00%
Coronavirus NL63	Hydrolysis Probe	243	99.21%	1.79%	0.00%	0.00%
Coronavirus HKU1	Sense Primer	232	99.92%	0.08%	0.00%	0.00%
Coronavirus HKU1	Antisense Primer	232	99.96%	0.04%	0.00%	0.00%
Coronavirus HKU1	Hydrolysis Probe	236	99.94%	0.06%	0.00%	0.00%
Parainfluenza Virus 1	Sense Primer	479	99.97%	0.03%	0.00%	0.00%
Parainfluenza Virus 1	Antisense Primer	479	99.94%	0.06%	0.00%	0.00%
Parainfluenza Virus 1	Hydrolysis Probe	486	97.45%	2.55%	0.00%	0.00%
Parainfluenza Virus 2	Sense Primer	91	99.65%	0.35%	0.00%	0.00%
Parainfluenza Virus 2	Antisense Primer	91	99.65%	0.35%	0.00%	0.00%

Parainfluenza Virus 2	Hydrolysis Probe	95	99.78%	0.22%	0.00%	0.00%
Parainfluenza Virus 3	Sense Primer	2050	98.52%	1.48%	0.00%	0.00%
Parainfluenza Virus 3	Antisense Primer	2050	98.52%	1.48%	0.00%	0.00%
Parainfluenza Virus 3	Hydrolysis Probe	2050	98.74%	1.26%	0.00%	0.00%
Parainfluenza Virus 4	Sense Primer	64	99.96%	0.04%	0.00%	0.00%
Parainfluenza Virus 4	Antisense Primer	64	99.96%	0.04%	0.00%	0.00%
Parainfluenza Virus 4	Hydrolysis Probe	62	99.98%	0.02%	0.00%	0.00%
Human Rhinovirus	Sense Primer	6453	96.45%	3.55%	0.00%	0.00%
Human Rhinovirus	Antisense Primer	6453	95.24%	4.76%	0.00%	0.00%
Human Rhinovirus	Hydrolysis Probe	6564	94.63%	4.37%	0.00%	0.00%
Enterovirus	Sense Primer	9217	95.41%	4.59%	0.00%	0.00%
Enterovirus	Antisense Primer	9217	95.41%	4.59%	0.00%	0.00%
Enterovirus	Hydrolysis Probe	9214	94.89%	5.11%	0.00%	0.00%
Adenovirus	Sense Primer	5422	98.23%	1.77%	0.00%	0.00%
Adenovirus	Antisense Primer	5422	98.23%	1.77%	0.00%	0.00%
Adenovirus	Hydrolysis Probe	5224	97.65%	2.35%	0.00%	0.00%
Human Bocavirus	Sense Primer	609	98.42%	1.58%	0.00%	0.00%
Human Bocavirus	Antisense Primer	609	98.42%	1.58%	0.00%	0.00%
Human Bocavirus	Hydrolysis Probe	643	98.63%	1.37%	0.00%	0.00%
Respiratory Syncytial Virus A	Sense Primer	4615	98.42%	1.58%	0.00%	0.00%
Respiratory Syncytial Virus A	Antisense Primer	4615	98.42%	1.58%	0.00%	0.00%
Respiratory Syncytial Virus A	Hydrolysis Probe	4618	97.46%	2.54%	0.00%	0.00%
Respiratory Syncytial Virus B	Sense Primer	8314	98.66%	1.34%	0.00%	0.00%
Respiratory Syncytial Virus B	Antisense Primer	8314	97.76%	2.24%	0.00%	0.00%
Respiratory Syncytial Virus B	Hydrolysis Probe	8509	98.12%	1.88%	0.00%	0.00%
Human Metapneumovirus A	Sense Primer	1502	97.56%	2.44%	0.00%	0.00%
Human Metapneumovirus A	Antisense Primer	1502	99.81%	0.19%	0.00%	0.00%
Human Metapneumovirus A	Hydrolysis Probe	1504	99.85%	0.15%	0.00%	0.00%
Human Metapneumovirus B	Sense Primer	1341	97.55%	2.45%	0.00%	0.00%
Human Metapneumovirus B	Antisense Primer	1341	97.55%	2.45%	0.00%	0.00%
Human Metapneumovirus B	Hydrolysis Probe	1104	99.28%	0.72%	0.00%	0.00%
Epstein–Barr Virus	Sense Primer	1212	99.76%	0.24%	0.00%	0.00%

Epstein–Barr Virus	Antisense Primer	1212	99.76%	0.24%	0.00%	0.00%
Epstein–Barr Virus	Hydrolysis Probe	1208	99.68%	032%	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%
Legionella pneumophila	Sense Primer	366	99.99%	0.01%	0.00%	0.00%
Legionella pneumophila	Antisense Primer	366	99.79%	0.21%	0.00%	0.00%
Legionella pneumophila	Hydrolysis Probe	370	99.99%	0.01%	0.00%	0.00%
Mycoplasma pneumoniae	Sense Primer	86	100.00%	0.00%	0.00%	0.00%
Mycoplasma pneumoniae	Antisense Primer	86	100.00%	0.00%	0.00%	0.00%
Mycoplasma pneumoniae	Hydrolysis Probe	86	100.00%	0.00%	0.00%	0.00%
Chlamydia pneumoniae	Sense Primer	93	99.13%	0.87%	0.00%	0.00%
Chlamydia pneumoniae	Antisense Primer	93	98.83%	1.27%	0.00%	0.00%
Chlamydia pneumoniae	Hydrolysis Probe	93	99.46%	0.54%	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%
Bordetella pertussis	Sense Primer	1070	99.89%	0.11%	0.00%	0.00%
Bordetella pertussis	Antisense Primer	1070	99.83%	0.17%	0.00%	0.00%
Bordetella pertussis	Hydrolysis Probe	1073	99.65%	0.35%	0.00%	0.00%
Bordetella parapertussis	Sense Primer	118	97.89%	2.11%	0.00%	0.00%
Bordetella parapertussis	Antisense Primer	118	97.87%	2.13%	0.00%	0.00%
Bordetella parapertussis	Hydrolysis Probe	118	96.12%	3.88%	0.00%	0.00%
Streptococcus pneumoniae	Sense Primer	387	99.97%	0.03%	0.00%	0.00%
Streptococcus pneumoniae	Antisense Primer	387	99.97%	0.03%	0.00%	0.00%
Streptococcus pneumoniae	Hydrolysis Probe	392	100.00%	0.00%	0.00%	0.00%
Moraxella catarrhalis	Sense Primer	485	100.00%	0.00%	0.00%	0.00%
Moraxella catarrhalis	Antisense Primer	485	100.00%	0.00%	0.00%	0.00%
Moraxella catarrhalis	Hydrolysis Probe	484	100.00%	0.00%	0.00%	0.00%
Staphylococcus aureus	Sense Primer	657	99.80%	0.20%	0.00%	0.00%
Staphylococcus aureus	Antisense Primer	657	99.80%	0.20%	0.00%	0.00%
Staphylococcus aureus	Hydrolysis Probe	655	99.70%	0.30%	0.00%	0.00%

Klebsiella pneumoniae	Sense Primer	785	99.55%	0.45%	0.00%	0.00%
Klebsiella pneumoniae	Antisense Primer	785	99.55%	0.45%	0.00%	0.00%
Klebsiella pneumoniae	Hydrolysis Probe	745	99.55%	0.45%	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%
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%
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%
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%
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%
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%
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%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab Pre-Plated Respiratory Pathogen 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 Respiratory Pathogen 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
SARS-CoV-2	ATCC VR-1986HK	1x
SARS-CoV-2 Delta	ATCC VR-3342HK	1x
SARS-CoV-2 Omicron	ATCC VR-3378HK	1x
Influenza A H1N1	Zeptometrix 0810036CF	1x
Influenza A H1N1pdm09	Zeptometrix 0810109CFJ	1x
Influenza A H3N2	Zeptometrix 0810252CF	3x
Influenza B	Zeptometrix 0810255CF	1x
Influenza B Victoria	Zeptometrix 0810258CF	3x
Influenza B Yamagata	Zeptometrix 0810256CF	3x
Coronavirus 229E	Zeptometrix 0810229CF	1x
Coronavirus OC43	Zeptometrix 0810024CF	1x
Coronavirus NL63	Zeptometrix 0810228CF	1x
Coronavirus HKU1	ATCC VR-3262SD	1x
Parainfluenza Virus 1	Zeptometrix 0810014CF	1x
Parainfluenza Virus 2	Zeptometrix 0810015CF	1x
Parainfluenza Virus 3	Zeptometrix 0810016CF	1x
Parainfluenza Virus 4A	Zeptometrix 0810060CF	1x
Parainfluenza Virus 4B	Zeptometrix 0810060BCF	3x
Human Rhinovirus A	Zeptometrix 0810012CFN	1x
Human Rhinovirus B	ATCC VR-284	3x
Human Enterovirus	Zeptometrix 0810300CF	1x
Adenovirus A	Zeptometrix 0810073CF	3x
Adenovirus B	Zeptometrix 0810062CF	1x
Adenovirus C	ATCC VR-1	1x
Adenovirus D	Zeptometrix 0810115CF	9x
Human Bocavirus	ATCC VR-3251SD	1x
Respiratory Syncytial Virus A	Zeptometrix 0810040ACF	1x
Respiratory Syncytial Virus B	Zeptometrix 0810040CF	1x
Human Metapneumovirus A1	Zeptometrix 0810161CF	1x
Human Metapneumovirus B1	Zeptometrix 0810158CF	3x

Epstein–Barr Virus	Zeptometrix 0810008CF	1x
Streptococcus pyogenes	Zeptometrix 0801512	1x
Legionella pneumophila	Zeptometrix 0801645	1x
Mycoplasma pneumoniae	Zeptometrix 0801579	1x
Chlamydophila pneumoniae	Zeptometrix 0804392	1x
Haemophilus influenzae	ATCC 33391	1x
Bordetella pertussis	ATCC 9797	1x
Streptococcus pneumoniae	ATCC 33400	1x
Moraxella catarrhalis	ATCC 25238	1x
Staphylococcus aureus	ATCC 12600	1x
Klebsiella pneumoniae	ATCC 13883	1x
mecA	ATCC BAA-2094	1x
mecC	ATCC BAA-2313	1x
Enterobacter cloacae	Zeptometrix 0801830	1x
Acinetobacter baumannii	ATCC 19606	1x
Klebsiella aerogenes	ATCC 13048	1x
Proteus mirabilis	Zeptometrix 0801544	1x
Pseudomonas aeruginosa	ATCC 27853	1x
Staphylococcus epidermidis	Zeptometrix 0804281	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	SARS-CoV-2	None	None	None
On-Panel	Influenza A	None	None	None
On-Panel	Influenza B	None	None	None

On-Panel	Coronavirus 229E	None	None	None
On-Panel	Coronavirus OC43	None	None	None
On-Panel	Coronavirus NL63	None	None	None
On-Panel	Coronavirus HKU1	None	None	None
On-Panel	Parainfluenza Virus 1	None	None	None
On-Panel	Parainfluenza Virus 2	None	None	None
On-Panel	Parainfluenza Virus 3	None	None	None
On-Panel	Parainfluenza Virus 4	None	None	None
On-Panel	Human Rhinovirus	None	None	None
On-Panel	Enterovirus	None	None	None
On-Panel	Adenovirus	None	None	None
On-Panel	Human Bocavirus 1/2/3/4	None	None	None
On-Panel	Respiratory Syncytial Virus A/B	None	None	None
On-Panel	Human Metapneumovirus	None	None	None
On-Panel	Epstein–Barr Virus	None	None	None
On-Panel	*Streptococcus pyogenes*	None	None	None
On-Panel	*Legionella pneumophila*	None	None	None
On-Panel	*Mycoplasma pneumoniae*	None	None	None
On-Panel	*Chlamydophila pneumoniae*	None	None	None
On-Panel	*Haemophilus influenzae*	None	None	None
On-Panel	*Bordetella pertussis*	None	None	None
On-Panel	*Streptococcus pneumoniae*	None	None	None
On-Panel	*Moraxella catarrhalis*	None	None	None
On-Panel	*Staphylococcus aureus*	None	None	None
On-Panel	*Klebsiella pneumoniae*	None	None	None
On-Panel	mecA	None	None	None
On-Panel	mecC	None	None	None
On-Panel	*Enterobacter cloacae*	None	None	None
On-Panel	*Acinetobacter baumannii*	None	None	None
On-Panel	*Klebsiella aerogenes*	None	None	None
On-Panel	*Proteus mirabilis*	None	None	None
On-Panel	*Pseudomonas aeruginosa*	None	None	None

On-Panel	*Staphylococcus epidermidis*	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	*Legionella longbeachae*	None	None	None
Off-Panel	*Stenotrophomonas maltophilia*	None	None	None
Off-Panel	*Neisseria meningitidis*	None	None	None
Off-Panel	*Neisseria elongata*	None	None	None
Off-Panel	*Acinetobacter calcoaceticus*	None	None	None
Off-Panel	*Bordetella bronchiseptica*	None	None	None
Off-Panel	*Bordetella hinzii*	None	None	None
Off-Panel	*Bacillus anthracis*	None	None	None
Off-Panel	*Bordetella holmesii*	None	None	None
Off-Panel	*Mycoplasma genitalium*	None	None	None
Off-Panel	*Ureaplasma urealyticum*	None	None	None
Off-Panel	*Ureaplasma parvum*	None	None	None
Off-Panel	*Streptococcus dysgalactiae*	None	None	None
Off-Panel	*Mycoplasma hominis*	None	None	None
Off-Panel	*Legionella bozemanae*	None	None	None
Off-Panel	*Coxiella burnetii*	None	None	None
Off-Panel	*Mycobacterium tuberculosis*	None	None	None
Off-Panel	*Arcanobacterium haemolyticum*	None	None	None
Off-Panel	*Chlamydia psittaci*	None	None	None
Off-Panel	*Streptococcus agalactiae*	None	None	None
Off-Panel	*Klebsiella oxytoca*	None	None	None
Off-Panel	*Bordetella avium*	None	None	None
Off-Panel	*Fusobacterium necrophorum*	None	None	None
Off-Panel	*Lactobacillus plantarum*	None	None	None
Off-Panel	*Lactobacillus acidophilus*	None	None	None
Off-Panel	*Neisseria gonorrhoeae*	None	None	None
Off-Panel	*Streptococcus salivarius*	None	None	None
Off-Panel	*Escherichia coli*	None	None	None

Off-Panel	*Serratia marcescens*	None	None	None
Off-Panel	*Legionella micdadei*	None	None	None
Off-Panel	*Corynebacterium striatum*	None	None	None
Off-Panel	*Leptospira interrogans*	None	None	None
Off-Panel	*Bordetella bronchiseptica*	None	None	None
Off-Panel	*Legionella feeleii*	None	None	None
Off-Panel	*Mycoplasma orale*	None	None	None
Off-Panel	*Aspergillus flavus*	None	None	None
Off-Panel	*Aspergillus fumigatus*	None	None	None
Off-Panel	*Blastomyces dermatitidis*	None	None	None
Off-Panel	*Candida albicans*	None	None	None
Off-Panel	*Cryptococcus neoformans*	None	None	None
Off-Panel	*Histoplasma capsulatum*	None	None	None
Off-Panel	*Pneumocystis jirovecii*	None	None	None
Off-Panel	Bat SARS-like Coronavirus	None	None	None
Off-Panel	MERS-CoV	None	None	None
Off-Panel	SARS	None	None	None
Off-Panel	Cytomegalovirus	None	None	None
Off-Panel	Herpes Simplex Virus 1	None	None	None
Off-Panel	Herpes Simplex Virus 2	None	None	None
Off-Panel	Human Herpes Virus 6	None	None	None
Off-Panel	Human Herpes Virus 7	None	None	None
Off-Panel	Measles Virus	None	None	None
Off-Panel	Mumps	None	None	None
Off-Panel	Astrovirus	None	None	None
Off-Panel	Rotavirus A	None	None	None
Off-Panel	HPV16	None	None	None
Off-Panel	HPV18	None	None	None
Off-Panel	Human Parechovirus	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 Respiratory Pathogen 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 Respiratory Pathogen 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 Pre-Plated Respiratory Pathogen Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
SARS-CoV-2	ATCC VR-1986HK	None
SARS-CoV-2 Delta	ATCC VR-3342HK	None
SARS-CoV-2 Omicron	ATCC VR-3378HK	None
Influenza A H1N1	Zeptometrix 0810036CF	None
Influenza A H1N1pdm09	Zeptometrix 0810109CFJ	None
Influenza A H3N2	Zeptometrix 0810252CF	None
Influenza B	Zeptometrix 0810255CF	None
Influenza B Victoria	Zeptometrix 0810258CF	None
Influenza B Yamagata	Zeptometrix 0810256CF	None
Coronavirus 229E	Zeptometrix 0810229CF	None
Coronavirus OC43	Zeptometrix 0810024CF	None
Coronavirus NL63	Zeptometrix 0810228CF	None
Coronavirus HKU1	ATCC VR-3262SD	None
Parainfluenza Virus 1	Zeptometrix 0810014CF	None
Parainfluenza Virus 2	Zeptometrix 0810015CF	None
Parainfluenza Virus 3	Zeptometrix 0810016CF	None
Parainfluenza Virus 4A	Zeptometrix 0810060CF	None
Parainfluenza Virus 4B	Zeptometrix 0810060BCF	None
Human Rhinovirus A	Zeptometrix 0810012CFN	None
Human Rhinovirus B	ATCC VR-284	None
Human Enterovirus	Zeptometrix 0810300CF	None

Adenovirus A	Zeptometrix 0810073CF	None
Adenovirus B	Zeptometrix 0810062CF	None
Adenovirus C	ATCC VR-1	None
Adenovirus D	Zeptometrix 0810115CF	None
Human Bocavirus	ATCC VR-3251SD	None
Respiratory Syncytial Virus A	Zeptometrix 0810040ACF	None
Respiratory Syncytial Virus B	Zeptometrix 0810040CF	None
Human Metapneumovirus A1	Zeptometrix 0810161CF	None
Human Metapneumovirus B1	Zeptometrix 0810158CF	None
Epstein Barr Virus	Zeptometrix 0810008CF	None
Streptococcus pyogenes	Zeptometrix 0801512	None
Legionella pneumophila	Zeptometrix 0801645	None
Mycoplasma pneumoniae	Zeptometrix 0801579	None
Chlamydophila pneumoniae	Zeptometrix 0804392	None
Haemophilus influenzae	ATCC 33391	None
Bordetella pertussis	ATCC 9797	None
Streptococcus pneumoniae	ATCC 33400	None
Moraxella catarrhalis	ATCC 25238	None
Staphylococcus aureus	ATCC 12600	None
Klebsiella pneumoniae	ATCC 13883	None
mecA	ATCC BAA-2094	None
mecC	ATCC BAA-2313	None
Enterobacter cloacae	Zeptometrix 0801830	None
Acinetobacter baumannii	ATCC 19606	None
Klebsiella aerogenes	ATCC 13048	None
Proteus mirabilis	Zeptometrix 0801544	None
Pseudomonas aeruginosa	ATCC 27853	None
Staphylococcus epidermidis	Zeptometrix 0804281	None

Table 14. Off-Panel organisms were tested for evaluation of ***MarinaBiolab Pre-Plated Respiratory Pathogen Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Acinetobacter calcoaceticus	ATCC 23055	None
Bordetella avium	ATCC 35086	None
Bordetella bronchiseptica	ATCC 10580	None
Bordetella holmesii	ATCC 700052	None
Chlamydia trachomatis	Zeptometrix 0801775	None
Escherichia coli	ATCC 25922	None
Haemophilus parainfluenzae	ATCC 9796	None
Legionella pneumophilia	Zeptometrix 0801530	None
Listeria monocytogenes	ATCC 19115	None
Mycobacterium tuberculosis	Zeptometrix 0801660	None
Mycoplasma genitalium	ATCC 33530D	None
Mycoplasma hominis	ATCC 27545-TTR	None
Neisseria gonorrhoeae	ATCC 19424	None
Neisseria meningitidis	ATCC 13090	None
Serratia marcescens	ATCC 29021	None
Staphylococcus haemolyticus	ATCC 29970	None
Ureaplasma urealyticum	ATCC 27618	None
Aspergillus flavus	Zeptometrix 0801598	None
Candida albicans	ATCC 10231	None
Pneumocystis jirovecii	ATCC PRA-159	None
Cryptococcus neoformans	ATCC MYA-4564	None
Cytomegalovirus	ATCC VR-977	None
Epstein Barr Virus	Zeptometrix 0810008CF	None
Measles	Zeptometrix 0810025CF	None
Mumps virus	Zeptometrix 0810079CF	None
Varicella-Zoster virus	Zeptometrix 0810026CF	None
Herpes Simplex Virus 1 (HSV1)	ATCC VR-1778	None
Herpes Simplex Virus 2 (HSV2)	Zeptometrix 0810217CF	None
Human Herpesvirus 6	Zeptometrix NATHHV6-STQ	None

Human Herpesvirus 7	Zeptometrix NATHHV7-ST	None
Human Parechovirus	Zeptometrix 0810145CF	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 Respiratory Pathogen 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 Respiratory Pathogen Panel PCR Kit***.

Table 15. Evaluation of potentially interfering substances on the ***MarinaBiolab Pre-Plated Respiratory Pathogen Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Human Blood	10% v/v	No Interference
Human Mucus (Sputum)	1 swab/mL sample	No Interference
Human Genomic DNA	20 ng/µL	No Interference
Competitive Microorganisms		
SARS-CoV-2	1.0E+05 unit/mL	No Interference
Influenza A	1.0E+05 unit/mL	No Interference
Influenza B	1.0E+05 unit/mL	No Interference
Coronavirus 229E	1.0E+05 unit/mL	No Interference
Coronavirus OC43	1.0E+05 unit/mL	No Interference
Coronavirus NL63	1.0E+05 unit/mL	No Interference
Coronavirus HKU1	1.0E+05 unit/mL	No Interference
Parainfluenza Virus 1	1.0E+05 unit/mL	No Interference
Parainfluenza Virus 2	1.0E+05 unit/mL	No Interference

Parainfluenza Virus 3	1.0E+05 unit/mL	No Interference
Parainfluenza Virus 4	1.0E+05 unit/mL	No Interference
Human Rhinovirus/Enterovirus	1.0E+05 unit/mL	No Interference
Adenovirus	1.0E+05 unit/mL	No Interference
Human Bocavirus 1/2/3/4	1.0E+05 unit/mL	No Interference
Respiratory Syncytial Virus A/B	1.0E+05 unit/mL	No Interference
Human Metapneumovirus	1.0E+05 unit/mL	No Interference
Epstein Barr Virus	1.0E+05 unit/mL	No Interference
Streptococcus pyogenes	1.0E+06 CFU/mL	No Interference
Legionella pneumophila	1.0E+06 CFU/mL	No Interference
Mycoplasma pneumoniae	1.0E+06 CFU/mL	No Interference
Chlamydophila pneumoniae	1.0E+06 CFU/mL	No Interference
Haemophilus influenzae	1.0E+06 CFU/mL	No Interference
Bordetella pertussis	1.0E+06 CFU/mL	No Interference
Streptococcus pneumoniae	1.0E+06 CFU/mL	No Interference
Moraxella catarrhalis	1.0E+06 CFU/mL	No Interference
Staphylococcus aureus	1.0E+06 CFU/mL	No Interference
Klebsiella pneumoniae	1.0E+06 CFU/mL	No Interference
mecA	1.0E+06 CFU/mL	No Interference
mecC	1.0E+06 CFU/mL	No Interference
Enterobacter cloacae	1.0E+06 CFU/mL	No Interference
Acinetobacter baumannii	1.0E+06 CFU/mL	No Interference
Klebsiella aerogenes	1.0E+06 CFU/mL	No Interference
Proteus mirabilis	1.0E+06 CFU/mL	No Interference
Pseudomonas aeruginosa	1.0E+06 CFU/mL	No Interference
Staphylococcus epidermidis	1.0E+06 CFU/mL	No Interference
Exogenous Substances		
Otrivine Adult Nasal Spray	1% v/v	No Interference
Tobramycin (systemic antibiotic)	1 mg/mL	No Interference
Amoxicillin + Penicillin + Cefadroxil + Erythromycin mixture	1% w/v	No Interference
Petroleum Jelly (Vaseline®)	1% w/v	No Interference
Rapivab (peramivir)	1% w/v	No Interference

Specimen Collection Materials		
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	100%	No Interference
Copan ESwab™ Sample Collection and Delivery System (Swab and Liquid Amies Medium)	100%	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 RT-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 RT-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 RT-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 RT-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 RT-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 RT-qPCR. If the issue persists, request a new sample, repeat the nucleic acid extraction and RT-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

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