RUO

INSTRUCTION FOR USE

Pre-Plated Gastrointestinal Panel PCR Kit

For Research Use Only

96

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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 Gastrointestinal 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	
Adenovirus F40/41	Astrovirus
Giardia lamblia	Rotavirus A
Entamoeba histolytica	*Salmonella spp.*
Cryptosporidium spp.	*Campylobacter spp.*
Clostridioides (Clostridium) difficile toxin A	*Shiga toxin-producing E. coli (STEC)*
Clostridioides (Clostridium) difficile toxin B	*Shigella/Enteroinvasive E. coli (EIEC)*
Vibrio vulnificus	*Enterotoxigenic E. coli (ETEC)*
Norovirus GI/GII	*Yersinia enterocolitica*
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 Gastrointestinal Panel PCR Kit*** consists of three main components:

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

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

Table 1. Kit components.

Component	Description	Quantity x Volume
		96 rxn PP-Gastro 010
Pre-Plated GIP Mix 1-8	Ready-to-use mix for RT-qPCR	96 Strips (7.5 µL)
PC-GIP	A mixture of non-infectious cDNA and DNA from artificial samples, including the targets listed in the table below	1 x 400 µL
NTC-GIP	DNase/RNase-Free Water	1 x 400 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
GIP Oligo Mix 1	-	**FAM**
	Human RNase P (IC)	**HEX/VIC/JOE**
	-	**ROX/Texas Red**
	Adenovirus F40/41	**CY5**
GIP Oligo Mix 2	*Giardia lamblia*	**FAM**
	Entamoeba histolytica	**HEX/VIC/JOE**
	Cryptosporidium spp.	**ROX/Texas Red**
	MS2 Bacteriophage (EC)	**CY5**
GIP Oligo Mix 3	-	**FAM**
	Clostridioides (Clostridium) difficile toxin B	**HEX/VIC/JOE**
	-	**ROX/Texas Red**
	Vibrio vulnificus	**CY5**
GIP Oligo Mix 4	Norovirus GI/GII	**FAM**
	Astrovirus	**HEX/VIC/JOE**
	-	**ROX/Texas Red**

	Rotavirus A	**CY5**
GIP Oligo Mix 5	*Campylobacter spp.*	**FAM**
	-	**HEX/VIC/JOE**
	Salmonella spp.	**ROX/Texas Red**
	-	**CY5**
GIP Oligo Mix 6	-	**FAM**
	Shiga toxin-producing E. coli (STEC)	**HEX/VIC/JOE**
	-	**ROX/Texas Red**
	Shigella/Enteroinvasive E. coli (EIEC)	**CY5**
GIP Oligo Mix 7	-	**FAM**
	Enterotoxigenic E. coli (ETEC)	**HEX/VIC/JOE**
	-	**ROX/Texas Red**
	Bacillus atrophaeus (EC)	**CY5**
GIP Oligo Mix 8	*Clostridioides (Clostridium) difficile toxin A*	**FAM**
	Yersinia enterocolitica	**HEX/VIC/JOE**
	-	**ROX/Texas Red**
	-	**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 Gastrointestinal 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 Gastrointestinal 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 1 strip for each sample or control.
- The orientations of Strip should be as shown below.

Strip

- Open carefully the strips and add 2.5 µL of the isolated sample or control to the corresponding wells.
- The final reaction mix volume is 10 µL.
- Re-cap the strips and **spin down** for 15 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. 4 empty strips for PC-Mix and NTC-Mix are included in the box.

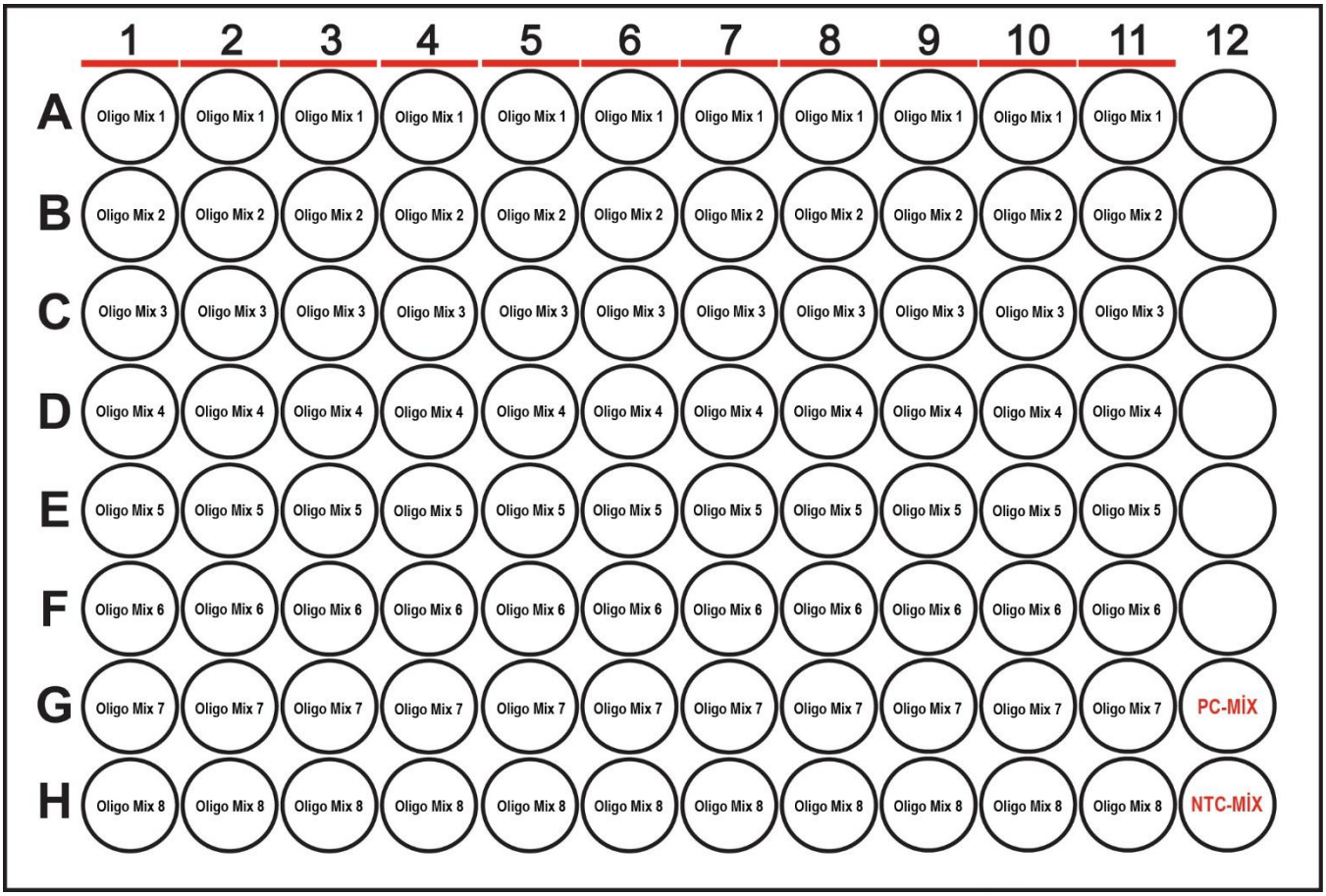

Table 3. Amplification profile.

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 Gastrointestinal 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 Gastrointestinal 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 Gastrointestinal 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
Adenovirus F40/41	Zeptometrix 0810084CF	4.8E+01 copies/mL	**20/20** 100%
Norovirus GI	ATCC VR-3234SD	5.1E+01 copies/mL	**20/20** 100%
Norovirus GII	ATCC VR-3235SD	6.2E+01 copies/mL	**20/20** 100%
Astrovirus	ATCC VR-1936	7.3E+01 copies/mL	**20/20** 100%
Rotavirus A	Zeptometrix 0810041CF	6.6E+01 copies/mL	**20/20** 100%
Vibrio vulnificus	Zeptometrix 0804349	2.2E+02 copies/mL	**20/20** 100%
Salmonella spp.	Zeptometrix 0804268	8.0E+01 copies/mL	**20/20** 100%
Campylobacter spp.	Zeptometrix 0804272	5.5E+01 copies/mL	**20/20** 100%
Shiga toxin-producing E. coli (STEC)	Zeptometrix 0801793	1.2E+02 copies/mL	**20/20** 100%
Shigella/Enteroinvasive E. coli (EIEC)	Zeptometrix 0801747	9.8E+01 copies/mL	**20/20** 100%
Enterotoxigenic E. coli (ETEC)	Zeptometrix 0801624DNA-10UG	8.0E+01 copies/mL	**20/20** 100%
Yersinia enterocolitica	Zeptometrix 0801734	1.4E+02 copies/mL	**20/20** 100%

Clostridioides (Clostridium) difficile toxin A	ATCC 9689	1.2E+02 copies/mL	**20/20** 100%
Clostridioides (Clostridium) difficile toxin B	ATCC 9689	9.5E+01 copies/mL	**20/20** 100%
Giardia lamblia	Zeptometrix 0801788	2.1E+02 copies/mL	**20/20** 100%
Entamoeba histolytica	Zeptometrix NATEHI(DS4)-GP	2.2E+02 copies/mL	**20/20** 100%
Cryptosporidium spp.	Zeptometrix 0801700	1.8E+02 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 Adenovirus F40/41, *Giardia lamblia, Entamoeba histolytica, Cryptosporidium spp., Yersinia enterocolitica, Vibrio vulnificus*, Norovirus GI/GII, Astrovirus, Rotavirus A, *Campylobacter spp., Salmonella spp., Shiga toxin-producing E. coli (STEC), Shigella/Enteroinvasive E. coli (EIEC), Enterotoxigenic E. coli (ETEC), Clostridioides (Clostridium) difficile toxin A,* and *Clostridioides (Clostridium) difficile toxin B* 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
Adenovirus F40/41	Sense Primer	221	100.00%	0.00%	0.00%	0.00%
Adenovirus F40/41	Antisense Primer	221	99.99%	0.005%	0.00%	0.00%
Adenovirus F40/41	Hydrolysis Probe	218	99.99%	0.005%	0.00%	0.00%

Norovirus GI	Sense Primer	3242	99.60%	0.40%	0.00%	0.00%
Norovirus GI	Antisense Primer	3242	98.42%	1.58%	0.00%	0.00%
Norovirus GI	Hydrolysis Probe	3242	99.20%	0.80%	0.00%	0.00%
Norovirus GII	Sense Primer	15453	98.46%	1.54%	0.00%	0.00%
Norovirus GII	Antisense Primer	15453	97.82%	2.18%	0.00%	0.00%
Norovirus GII	Hydrolysis Probe	15648	97.98%	2.02%	0.00%	0.00%
Astrovirus	Sense Primer	708	100.00%	0.00%	0.00%	0.00%
Astrovirus	Antisense Primer	708	100.00%	0.00%	0.00%	0.00%
Astrovirus	Hydrolysis Probe	1136	93.69%	6.16%	0.15%	0.00%
Rotavirus A	Sense Primer	4340	97.70%	2.30%	0.00%	0.00%
Rotavirus A	Antisense Primer	4340	97.97%	1.93%	0.10%	0.00%
Rotavirus A	Hydrolysis Probe	4644	98.03%	1.97%	0.00%	0.00%
Campylobacter spp.	Sense Primer	628	99.99%	0.005%	0.00%	0.00%
Campylobacter spp.	Antisense Primer	628	99.99%	0.005%	0.00%	0.00%
Campylobacter spp.	Hydrolysis Probe	634	99.99%	0.005%	0.005%	0.00%
Salmonella spp.	Sense Primer	4256	98.24%	1.76%	0.00%	0.00%
Salmonella spp.	Antisense Primer	4256	98.46%	1.54%	0.00%	0.00%
Salmonella spp.	Hydrolysis Probe	4324	97.88%	2.12%	0.00%	0.00%
Vibrio vulnificus	Sense Primer	53	100.00%	0.00%	0.00%	0.00%
Vibrio vulnificus	Antisense Primer	53	100.00%	0.00%	0.00%	0.00%
Vibrio vulnificus	Hydrolysis Probe	52	100.00%	0.00%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin A	Sense Primer	227	99.99%	0.005%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin A	Antisense Primer	227	99.99%	0.005%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin A	Hydrolysis Probe	227	99.99%	0.005%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin B	Sense Primer	250	100.00%	0.00%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin B	Antisense Primer	250	99.80%	0.20%	0.00%	0.00%
Clostridioides (Clostridium) difficile toxin B	Hydrolysis Probe	241	100.00%	0.00%	0.00%	0.00%
Yersinia enterocolitica	Sense Primer	37	100.00%	0.00%	0.00%	0.00%
Yersinia enterocolitica	Antisense Primer	37	100.00%	0.00%	0.00%	0.00%
Yersinia enterocolitica	Hydrolysis Probe	38	99.99%	0.005%	0.00%	0.00%

Shiga toxin-producing E. coli (STEC) (stx1)	Sense Primer	483	99.99%	0.01%	0.00%	0.00%
Shiga toxin-producing E. coli (STEC) (stx1)	Antisense Primer	483	99.99%	0.01%	0.00%	0.00%
Shiga toxin-producing E. coli (STEC) (stx1)	Hydrolysis Probe	484	92.74%	7.26%	0.00%	0.00%
Shiga toxin-producing E. coli (STEC) (stx2)	Sense Primer	1235	96.48%	3.52%	0.00%	0.00%
Shiga toxin-producing E. coli (STEC) (stx2)	Antisense Primer	1235	97.86%	2.14%	0.00%	0.00%
Shiga toxin-producing E. coli (STEC) (stx2)	Hydrolysis Probe	1348	97.84%	2.12%	0.00%	0.00%
Shigella/Enteroinvasive E. coli (EIEC)	Sense Primer	444	99.99%	0.005%	0.00%	0.00%
Shigella/Enteroinvasive E. coli (EIEC)	Antisense Primer	444	99.99%	0.005%	0.00%	0.00%
Shigella/Enteroinvasive E. coli (EIEC)	Hydrolysis Probe	440	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (lt)	Sense Primer	114	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (lt)	Antisense Primer	114	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (lt)	Hydrolysis Probe	114	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (st)	Sense Primer	79	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (st)	Antisense Primer	79	100.00%	0.00%	0.00%	0.00%
Enterotoxigenic E. coli (ETEC) (st)	Hydrolysis Probe	78	100.00%	0.00%	0.00%	0.00%
Giardia lamblia	Sense Primer	3410	99.24%	0.76%	0.00%	0.00%
Giardia lamblia	Antisense Primer	3410	99.20%	0.80%	0.00%	0.00%
Giardia lamblia	Hydrolysis Probe	3770	99.14%	0.86%	0.00%	0.00%
Entamoeba histolytica	Sense Primer	206	99.99%	0.005%	0.00%	0.00%
Entamoeba histolytica	Antisense Primer	206	99.99%	0.005%	0.00%	0.00%
Entamoeba histolytica	Hydrolysis Probe	231	99.99%	0.005%	0.00%	0.00%
Cryptosporidium spp.	Sense Primer	301	96.46%	3.54%	0.00%	0.00%
Cryptosporidium spp.	Antisense Primer	301	97.40%	2.60%	0.00%	0.00%
Cryptosporidium spp.	Hydrolysis Probe	310	98.46%	1.54%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab Pre-Plated Gastrointestinal 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 Gastrointestinal 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
Adenovirus F40/41	Zeptometrix 0810084CF	1x
Norovirus GI	ATCC VR-3234SD	1x
Norovirus GII	ATCC VR-3235SD	1x
Astrovirus	ATCC VR-1936	1x
Rotavirus A	Zeptometrix 0810041CF	1x
Vibrio vulnificus	Zeptometrix 0804349	1x
Salmonella spp.	Zeptometrix 0804268	1x
Campylobacter spp.	Zeptometrix 0804272	1x
Shiga toxin-producing E. coli (STEC)	Zeptometrix 0801793	1x
Shigella/Enteroinvasive E. coli (EIEC)	Zeptometrix 0801747	1x
Enterotoxigenic E. coli (ETEC)	Zeptometrix 0801624DNA-10UG	1x
Yersinia enterocolitica	Zeptometrix 0801734	1x
Clostridioides (Clostridium) difficile toxin A	ATCC 9689	1x
Clostridioides (Clostridium) difficile toxin B	ATCC 9689	1x
Giardia lamblia	Zeptometrix 0801788	1x
Entamoeba histolytica	Zeptometrix NATEHI(DS4)-GP	1x
Cryptosporidium spp.	Zeptometrix 0801700	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	Adenovirus F40/41	None	None	None
On-Panel	Norovirus GI/GII	None	None	None
On-Panel	Astrovirus	None	None	None
On-Panel	Rotavirus A	None	None	None
On-Panel	*Vibrio vulnificus*	None	None	None
On-Panel	*Salmonella spp.*	None	None	None
On-Panel	*Campylobacter spp.*	None	None	None
On-Panel	*Shiga toxin-producing E. coli (STEC)*	None	None	None
On-Panel	*Shigella/Enteroinvasive E. coli (EIEC)*	None	None	None
On-Panel	*Enterotoxigenic E. coli (ETEC)*	None	None	None
On-Panel	*Yersinia enterocolitica*	None	None	None
On-Panel	*Clostridioides (Clostridium) difficile toxin A*	None	None	None
On-Panel	*Clostridioides (Clostridium) difficile toxin B*	None	None	None
On-Panel	*Giardia lamblia*	None	None	None
On-Panel	*Entamoeba histolytica*	None	None	None
On-Panel	*Cryptosporidium spp.*	None	None	None
Off-Panel	*Abiotrophia defectivia*	None	None	None
Off-Panel	*Acinetobacter baumannii*	None	None	None
Off-Panel	*Acinetobacter lwoffii*	None	None	None
Off-Panel	*Aeromonas hydrophila*	None	None	None
Off-Panel	*Alcaligenes faecalis*	None	None	None
Off-Panel	*Anaerococcus tetradius*	None	None	None
Off-Panel	*Arcobacter butzleri*	None	None	None
Off-Panel	*Arcobacter cryaerophilus*	None	None	None
Off-Panel	*Bacillus cereus*	None	None	None
Off-Panel	*Bacteroides fragilis*	None	None	None
Off-Panel	*Bacteroides thetaiotaomicron*	None	None	None

Off-Panel	*Bacteroides vulgatus*	None	None	None
Off-Panel	*Bifidobacterium adolescentisa*	None	None	None
Off-Panel	*Bifidobacterium bifiduma*	None	None	None
Off-Panel	*Bifidobacterium longuma*	None	None	None
Off-Panel	*Cedecea davisaeb*	None	None	None
Off-Panel	*Chlamydia trachomatis*	None	None	None
Off-Panel	*Citrobacter amalonaticus*	None	None	None
Off-Panel	*Citrobacter freundii*	None	None	None
Off-Panel	*Clostridium acetobutylicum*	None	None	None
Off-Panel	*Clostridium botulinum*	None	None	None
Off-Panel	*Clostridium difficile non-toxigenic*	None	None	None
Off-Panel	*Clostridium histolyticum*	None	None	None
Off-Panel	*Clostridium methylpentosum*	None	None	None
Off-Panel	*Clostridium novyi*	None	None	None
Off-Panel	*Clostridium perfringens*	None	None	None
Off-Panel	*Clostridium ramosum*	None	None	None
Off-Panel	*Clostridium septicum*	None	None	None
Off-Panel	*Clostridium sordellii*	None	None	None
Off-Panel	*Clostridium tetani*	None	None	None
Off-Panel	*Collinsella aerofaciens*	None	None	None
Off-Panel	*Corynebacterium genitalium*	None	None	None
Off-Panel	*Desulfovibrio piger*	None	None	None
Off-Panel	*Diffusely adherent E.coli*	None	None	None
Off-Panel	*Edwardsiella tarda*	None	None	None
Off-Panel	*Egglerthella lenta*	None	None	None
Off-Panel	*Enterobacter aerogenes*	None	None	None
Off-Panel	*Enterobacter cloacae*	None	None	None
Off-Panel	*Enterococcus faecalis*	None	None	None
Off-Panel	*Enterococcus faecium*	None	None	None
Off-Panel	*Escherichia blattae*	None	None	None
Off-Panel	*Escherichia fergusonii*	None	None	None
Off-Panel	*Escherichia hermannii*	None	None	None

Off-Panel	*Escherichia vulneris*	None	None	None
Off-Panel	*Eubacterium cylindroides*	None	None	None
Off-Panel	*Eubacterium rectale*	None	None	None
Off-Panel	*Faecalibacterium prausnitzii*	None	None	None
Off-Panel	*Fusobacterium varium*	None	None	None
Off-Panel	*Gardnerella vaginalis*	None	None	None
Off-Panel	*Gemella morbillorum*	None	None	None
Off-Panel	*Haemophilus influenzae*	None	None	None
Off-Panel	*Hafnia alveib*	None	None	None
Off-Panel	*Helicobacter fennelliae*	None	None	None
Off-Panel	*Helicobacter pylori*	None	None	None
Off-Panel	*Klebsiella oxytoca*	None	None	None
Off-Panel	*Klebsiella pneumoniae*	None	None	None
Off-Panel	*Lactobacillus acidophilus*	None	None	None
Off-Panel	*Lactobacillus reuteri*	None	None	None
Off-Panel	*Lactococcus lactis*	None	None	None
Off-Panel	*Leminorella grimontii*	None	None	None
Off-Panel	*Listeria monocytogenes*	None	None	None
Off-Panel	*Megamonas hypermegale*	None	None	None
Off-Panel	*Megasphaeara elsdenii*	None	None	None
Off-Panel	*Methanobrevibacter smithii*	None	None	None
Off-Panel	*Morganella morganii*	None	None	None
Off-Panel	*Peptoniphilus asaccharolyticus*	None	None	None
Off-Panel	*Peptostreptococcus anaerobius*	None	None	None
Off-Panel	*Photobacterium damselae*	None	None	None
Off-Panel	*Porphyromonas asaccharolytica*	None	None	None
Off-Panel	*Prevotella melaninogenica*	None	None	None
Off-Panel	*Proteus mirabilis*	None	None	None
Off-Panel	*Proteus penneri*	None	None	None
Off-Panel	*Proteus vulgaris*	None	None	None
Off-Panel	*Provedencia alcalifaciens*	None	None	None
Off-Panel	*Pseudomonas aeruginosa*	None	None	None

Off-Panel	*Ruminococcus bromiia*	None	None	None
Off-Panel	*Ruminococcus flavefaciensa*	None	None	None
Off-Panel	*Ruminococcus obeuma*	None	None	None
Off-Panel	*Selenomonas ruminantium*	None	None	None
Off-Panel	*Serratia liquefaciens*	None	None	None
Off-Panel	*Serratia marcescens*	None	None	None
Off-Panel	*Shewanella algae*	None	None	None
Off-Panel	*Staphylococcus aureus*	None	None	None
Off-Panel	*Staphylococcus epidermidis*	None	None	None
Off-Panel	*Stenotrophomonas maltophilia*	None	None	None
Off-Panel	*Streptococcus agalactiae*	None	None	None
Off-Panel	*Streptococcus intermedius*	None	None	None
Off-Panel	*Streptococcus pyogenes*	None	None	None
Off-Panel	*Streptococcus salivarius*	None	None	None
Off-Panel	*Streptococcus suis*	None	None	None
Off-Panel	*Trabulsiella guamensis*	None	None	None
Off-Panel	*Veillonella parvula*	None	None	None
Off-Panel	*Yersinia bercovieri*	None	None	None
Off-Panel	*Yersinia frederiksenii*	None	None	None
Off-Panel	*Yersinia intermedia*	None	None	None
Off-Panel	*Yersinia mollaretii*	None	None	None
Off-Panel	*Yersinia pseudotuberculosis*	None	None	None
Off-Panel	*Yersinia rohdei*	None	None	None
Off-Panel	*Ancylostoma duodenale*	None	None	None
Off-Panel	*Ascaris lumbricoides*	None	None	None
Off-Panel	*Aspergillus fumigatus*	None	None	None
Off-Panel	*Babesia microti*	None	None	None
Off-Panel	*Balantidium coli*	None	None	None
Off-Panel	*Blastocystis hominis*	None	None	None
Off-Panel	*Candida albicans*	None	None	None
Off-Panel	*Candida catenulate*	None	None	None
Off-Panel	*Chilomastix mesnili*	None	None	None

Off-Panel	*Conidiobolus lachnodes*	None	None	None
Off-Panel	*Conidiobolus lobatus*	None	None	None
Off-Panel	*Dientamoeba fragilis*	None	None	None
Off-Panel	*Encephalitozoon hellem*	None	None	None
Off-Panel	*Encephalitozoon intestinalis*	None	None	None
Off-Panel	*Endolimax nana*	None	None	None
Off-Panel	*Entamoeba coli*	None	None	None
Off-Panel	*Entamoeba gingivalis*	None	None	None
Off-Panel	*Entamoeba hartmanni*	None	None	None
Off-Panel	*Entamoeba moshkovskii*	None	None	None
Off-Panel	*Entamoeba polecki*	None	None	None
Off-Panel	*Enterobius vermicularis*	None	None	None
Off-Panel	*Enteromonas hominis*	None	None	None
Off-Panel	*Giardia muris*	None	None	None
Off-Panel	*Isospora belli*	None	None	None
Off-Panel	*Necator americanus*	None	None	None
Off-Panel	*Penicillium marneffei*	None	None	None
Off-Panel	*Pentatrichomonas hominis*	None	None	None
Off-Panel	*Saccharomyces boulardi*	None	None	None
Off-Panel	*Saccharomyces cerevisiae*	None	None	None
Off-Panel	*Schistosoma mansoni*	None	None	None
Off-Panel	*Toxoplasma gondii*	None	None	None
Off-Panel	*Trichomonas tenax*	None	None	None
Off-Panel	Adenovirus A	None	None	None
Off-Panel	Adenovirus B	None	None	None
Off-Panel	Adenovirus C	None	None	None
Off-Panel	Adenovirus D	None	None	None
Off-Panel	Adenovirus E	None	None	None
Off-Panel	Adenovirus G	None	None	None
Off-Panel	Astrovirus variant MLB	None	None	None
Off-Panel	Astrovirus variant VA1	None	None	None
Off-Panel	Bocavirus Type 1	None	None	None

Off-Panel	Cytomegalovirus	None	None	None
Off-Panel	Echovirus 6	None	None	None
Off-Panel	Enterovirus 68	None	None	None
Off-Panel	Hepatitis A	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 Gastrointestinal 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 Gastrointestinal 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 Gastrointestinal Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Adenovirus F40/41	Zeptometrix 0810084CF	None
Norovirus GI	ATCC VR-3234SD	None
Norovirus GII	ATCC VR-3235SD	None
Astrovirus	ATCC VR-1936	None
Rotavirus A	Zeptometrix 0810041CF	None
Vibrio vulnificus	Zeptometrix 0804349	None
Salmonella spp.	Zeptometrix 0804268	None
Campylobacter spp.	Zeptometrix 0804272	None
Shiga toxin-producing E. coli (STEC)	Zeptometrix 0801793	None
Shigella/Enteroinvasive E. coli (EIEC)	Zeptometrix 0801747	None
Enterotoxigenic E. coli (ETEC)	Zeptometrix 0801624DNA-10UG	None
Yersinia enterocolitica	Zeptometrix 0801734	None
Clostridioides (Clostridium) difficile toxin A	ATCC 9689	None
Clostridioides (Clostridium) difficile toxin B	ATCC 9689	None
Giardia lamblia	Zeptometrix 0801788	None
Entamoeba histolytica	Zeptometrix NATEHI(DS4)-GP	None

Cryptosporidium spp.	Zeptometrix 0801700	None

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

Organism	Isolate ID/Source	Cross Reactivity Detected
Abiotrophia defectivia	ATCC 700209	None
Acinetobacter baumannii	Zeptometrix 801597	None
Acinetobacter lwoffii	Zeptometrix 801909	None
Aeromonas hydrophila	Zeptometrix 804098	None
Alcaligenes faecalis	Zeptometrix 801995	None
Anaerococcus tetradius	ATCC 35098	None
Arcobacter butzleri	ATCC 49616	None
Arcobacter cryaerophilus	ATCC 43158	None
Bacillus cereus	Zeptometrix 801823	None
Bacteroides fragilis	Zeptometrix 801583	None
Bacteroides thetaiotaomicron	Zeptometrix 801743	None
Bifidobacterium adolescentisa	Zeptometrix 801998	None
Bifidobacterium longuma	Zeptometrix 804047	None
Cedecea davisaeb	ATCC 33431	None
Chlamydia trachomatis	Zeptometrix 804400	None
Citrobacter amalonaticus	Zeptometrix 801718	None
Citrobacter freundii	Zeptometrix 801563	None
Clostridium acetobutylicum	ATCC 824	None
Clostridium difficile non-toxigenic	Zeptometrix 804105	None
Clostridium histolyticum	Zeptometrix 804054	None
Clostridium methylpentosum	ATCC 43829	None
Clostridium novyi	Zeptometrix 804056	None
Clostridium perfringens	Zeptometrix 801585	None
Clostridium ramosum	Zeptometrix 804058	None
Clostridium septicum	Zeptometrix 801885	None
Clostridium sordellii	Zeptometrix 801587	None
Clostridium tetani	Zeptometrix 804063	None

Collinsella aerofaciens	Zeptometrix 804064	None
Corynebacterium genitalium	Zeptometrix 804108	None
Desulfovibrio piger	ATCC 29098	None
Edwardsiella tarda	Zeptometrix 804065	None
Egglerthella lenta	Zeptometrix 804066	None
Enterobacter cloacae	Zeptometrix 801830	None
Enterococcus faecalis	Zeptometrix 804216	None
Enterococcus faecium	Zeptometrix 804328	None
Escherichia fergusonii	Zeptometrix 804113	None
Escherichia hermannii	Zeptometrix 804068	None
Eubacterium cylindroides	ATCC 27805	None
Eubacterium rectale	ATCC 33656	None
Faecalibacterium prausnitzii	ATCC 27768	None
Fusobacterium varium	Zeptometrix 804069	None
Gardnerella vaginalis	Zeptometrix 801894	None
Gemella morbillorum	Zeptometrix 804253	None
Haemophilus influenzae	Zeptometrix 801680	None
Hafnia alveib	ATCC 13337	None
Helicobacter pylori	Zeptometrix 804383	None
Klebsiella oxytoca	Zeptometrix 801881	None
Klebsiella pneumoniae	Zeptometrix 804295	None
Lactobacillus acidophilus	Zeptometrix 801540	None
Lactobacillus reuteri	Zeptometrix 804322	None
Lactococcus lactis	Zeptometrix 804157	None
Leminorella grimontii	Zeptometrix 804070	None
Listeria monocytogenes	Zeptometrix 804339	None
Megamonas hypermegale	ATCC 25560	None
Megasphaeara elsdenii	ATCC 25940	None
Morganella morganii	Zeptometrix 804010	None
Peptoniphilus asaccharolyticus	Zeptometrix 804245	None
Peptostreptococcus anaerobius	Zeptometrix 804012	None
Photobacterium damselae	ATCC 33539	None

Porphyromonas asaccharolytica	ATCC 27908	None
Prevotella melaninogenica	Zeptometrix 804292	None
Proteus mirabilis	Zeptometrix 801544	None
Proteus penneri	Zeptometrix 804442	None
Proteus vulgaris	Zeptometrix 801898	None
Provedencia alcalifaciens	Zeptometrix 804079	None
Pseudomonas aeruginosa	Zeptometrix 801908	None
Ruminococcus bromiia	ATCC 27255	None
Selenomonas ruminantium	ATCC 12561	None
Serratia liquefaciens	Zeptometrix 804207	None
Serratia marcescens	Zeptometrix 801723	None
Shewanella algae	Zeptometrix 804207	None
Staphylococcus aureus	Zeptometrix 804275	None
Staphylococcus epidermidis	Zeptometrix 804281	None
Stenotrophomonas maltophilia	Zeptometrix 801569	None
Streptococcus agalactiae	Zeptometrix 801556	None
Streptococcus intermedius	Zeptometrix 801895	None
Streptococcus pyogenes	Zeptometrix 801512	None
Aspergillus fumigatus	Zeptometrix 801716	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 Gastrointestinal 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 Gastrointestinal Panel PCR Kit***.

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

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Human Blood	10% v/v	No Interference
Fatty Acids (Palmitic Acid)	10% v/v	No Interference
Human Urine	-	No Interference
Human Stool	-	No Interference
Competitive Microorganisms		
Adenovirus F40/41	1.0E+05 unit/mL	No Interference
Norovirus GI	1.0E+05 unit/mL	No Interference
Norovirus GII	1.0E+05 unit/mL	No Interference
Astrovirus	1.0E+05 unit/mL	No Interference
Rotavirus A	1.0E+05 unit/mL	No Interference
Vibrio vulnificus	1.0E+06 CFU/mL	No Interference
Salmonella spp.	1.0E+06 CFU/mL	No Interference
Campylobacter spp.	1.0E+06 CFU/mL	No Interference
Shiga toxin-producing E. coli (STEC)	1.0E+06 CFU/mL	No Interference
Shigella/Enteroinvasive E. coli (EIEC)	1.0E+06 CFU/mL	No Interference
Enterotoxigenic E. coli (ETEC)	1.0E+06 CFU/mL	No Interference
Yersinia enterocolitica	1.0E+06 CFU/mL	No Interference
Clostridioides (Clostridium) difficile toxin A	1.0E+06 CFU/mL	No Interference
Clostridioides (Clostridium) difficile toxin B	1.0E+06 CFU/mL	No Interference
Giardia lamblia	1.0E+06 CFU/mL	No Interference
Entamoeba histolytica	1.0E+06 CFU/mL	No Interference
Cryptosporidium spp.	1.0E+06 CFU/mL	No Interference
Exogenous Substances		
Bacitracin	1% w/v	No Interference
Nystatin	1% w/v	No Interference
Glycerin	1% w/v	No Interference
Magnesium hydroxide	1% w/v	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

Tel: +1 510 579-5802

e-mail customer care: accounting@marinabiolab.com

e-mail Technical Support: rd@marinabiolab.com

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715 Discovery Blvd, suite 309 Cedar Park, TX 78613

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