GI Panel Real Time PCR Detection QP2166

INTENDED USE

The Gastrointestinal Panel Real Time PCR detection kit is a qualitative test intended for analyzing unpreserved stool samples in Cary-Blair transport medium taken from patients suspected of gastrointestinal infection for the presence of viral, parasitic or bacterial nucleic acids

SUMMARY AND EXPLANATION OF THE TEST

The assay includes the following respiratory tract bacteria targets:
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BACTERIA:

- *Campylobacter (C. jejuni / C. coli))*
- *Salmonella*
- *Yersinia enterocolitica*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*

DIARRHEAGENIC ESCHERICHIA COLI/SHIGELLA:

- *Enteroaggregative E. coli* (EAEC)
- *Enteropathogenic E. coli* (EPEC)
- Shigella/enteroinvasive E. Coli (EIEC)
- *Shiga-like toxin-producing E. coli* (STEC)
- Enterotoxigenic Escherichia coli(ETEC)
- Enterohemorrhagic E. Coli EHEC
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PARASITES:

- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

VIRUSES:

- Adenovirus F40/41
- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus (I, II, IV, and V)

PRINCIPLE

The primers and probes are selected from the regions of *targeted conserved regions of the above bacteria and viruses*. The primer/probe sets are designed for specific detection of above regions are separated into six multiplex qPCR setups. An additional primer/probe set to detect RNase P gene (as an internal control) is also included. DNA/RNA extracted from stool specimens is amplified in a Real-Time PCR Thermocycler.The fluorescent signal is emitted when the 5' nuclease active domain of Taq polymerase degrades the probe anneals to target sequence between the forward and the reverse primers. During each cycle, the fluorescence intensity is increased with the additional reporter dye molecules released from their respective probes.
The *GI Panel Assay* also contains a positive control (Plasmid DNA) and a negative control for testing process monitoring.

REAQENTS AND MATERIALS PROVIDED

Item	Component	Quantity	
		100 Tests / Box	1000 Tests / Box
1	GI PCR Mix 1 GI PCR Mix 2 GI PCR Mix 3 GI PCR Mix 4 GI PCR Mix 5 GI PCR Mix 6 GI PCR Mix 7	1.5 mL each	15 mL each
3	Positive Control	100 µL/tube × 1	500 µL/ tube × 1
4	Negative Control	100 µL/tube × 1	500 µL/ tube × 1

MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE)***, FAM, ROX and Cy5 channels.
2. Specimen Collection Containers
3. DNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex
7. High-speed centrifuge
8. Micropipette (0.5-2 µL, 1-10 µL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For use by professionals specifically trained in nucleic acid amplification techniques and *in vitro* diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposable gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should start from the DNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a DNA Extraction Kit prior testing. Do not perform a qPCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 12 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: stool sample in Cary-Blair stool culture transport medium.
Specimen preservation: process specimens for DNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for longer storage, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

To calculate the number (n) of PCR reaction tubes (n= number of specimens to be tested + number of positive controls + number of negative controls),

For panel 1&2: add n x14 µL of PCR Mix and n x 1 µL of RT-PCR Enzyme Mix to a clean tube, vortex to mix well, centrifuge and aliquot 15 µL of the mix into each PCR reaction tube.

For panel 3,4,5 and 6: add nx15 µL of PCR Mix and aliquot 15 µL of the mix into each PCR reaction tube.

B. Addition of sample

Add 5 µL of RNA/DNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, mix well and spin the mixture briefly.

PCR Reaction Preparation:

Component	Volume per test
PCR Mix	15 µL
Negative Control / Positive Control / or RNA of specimen to be tested	5 µL
Total Reaction Volume	20 µL

C. PCR amplification (at Amplification Area)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimens on the Thermocycler software.
2. Enter the PCR reaction volume: 20 µL.
3. Select fluorescence detection channels: JOE, FAM, Rox and Cy5 channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Reverse transcription	5 minutes	50 ºC
1	Initial denaturation	2 minutes	95 ºC
40	Denaturation	5 seconds	95 ºC
	Annealing/Extension	20 seconds	60 ºC

QUALITY CONTROL

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.
NC (negative control) material: no obvious amplification curve for Cy5 and FAM detection channels, and no obvious amplification curve for JOE and ROX channels.
PC (positive control) material: obvious amplification curves for Cy5 and FAM detection channels (Ct value ≤37), and obvious amplification curves for JOE and ROX channels.
Pay attention that the above requirements must be met each time when the experiment is performed; otherwise, the experiment is considered invalid

Multiplex qPCR setup 1

Channel	Bacteria	Results Interpretation
JOE	Astrovirus	
Fam	Adenovirus	
ROX	Rotavirus	
Cy5	Noravirus G1	

Multiplex qPCR setup 2

Channel	Bacteria	Results Interpretation
Cy5	Sapovirus	
FAM	Norovirus GII	
ROX	aaiC	
JOE	TcdB	

Multiplex qPCR setup 3

Channel	Bacteria	Results Interpretation
JOE	Yersinia enterocolitica	
FAM	Vibrio parahaemolyticus	
ROX	Cryptosporidium parvum	
Cy5	Cyclospora cayetanensis	

Multiplex qPCR setup 4

Channel	Bacteria	Results Interpretation
JOE	Campylobacte	
FAM	Salmonella enterica	
ROX	Giardia lamblia	
Cy5	RNase P	

Multiplex qPCR setup 5

Channel	Bacteria	Results Interpretation
JOE	eae	
FAM	stx2	
ROX	aggR	
Cy5	ipaH	

Multiplex qPCR setup 6

Channel	Bacteria	Results Interpretation
JOE	stx1	
FAM	uidA	
ROX	Vibrio vulnificus	
Cy5	Entamoeba	

ultiplex qPCR setup 7

Channel	Bacteria	Results Interpretation
FAM	STa	
ROX	STb	
LT	Cy5	

O157	VIC	

Interpretation of E.coli results,

•If uid is negative, it is negative for E coli;
•If uid is positive and ipah is positive, EIEC or Shigell is present;
•If uid is positive, and aggR or aaiC is positive, and stx1 and stx2 both negative, EAEC is present;
•If uid is positive, aggR and aaiC and eae-EBP1 are negative, and stx1 or stx2 positive,
STEC is present.
•If uid is positive, and aggR or aaiC is positive, and stx1 or stx2 positive, EAggSTEC (STEC with aggregates) is present.
•If uid is positive, and eae is positive, and stx1 and stx2 both negative, EPEC is present;
•If uid is positive, and eae is positive, and stx1 or stx2 positive, EHEC (one type of STEC) is present.

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
Thermofisher	QuantStudio 5, 7-plex, 12 K
BioRad	CFX 96 and 384

PRODUCT PERFORMANCE
Product analysis performance evaluation results:
1. The analytical sensitivity of this kit is 200 copies/mL.
2. Cross-reaction: no cross-reaction with other pathogens.

LIMITATIONS OF TEST
1. This test provides a presumptive diagnosis of GI pathogen infection. Negative test results do not preclude pathogen infection. All test results should be evaluated by healthcare professionals in the context of clinical symptoms, epidemiological information, patient history, and other diagnostic test results as the basis for patient management decisions.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; virus variation, PCR inhibitors , etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with respiratory specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper RNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.
8. Cross-contamination by samples containing high copies of GI pathogens, particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit component

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 597-0602 or visit our website at www.biomiga.com

Symbols used

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	*In vitro* diagnostic medical device
	Meet the requirements of EC Directive 98/79/EC
	Catalogue number
	Batch code
	The number of test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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Table for interpretation of *E.coli* results

uid	**ipah**	**aggR / aaiC**	**eae**	**stx1**	**stx2**	**STa/ STb/ LT**	**Z3276**	**Interpretation**
Negative	-	-	-	-	-	-	-	**Negative for *E. coli***
Positive	-	-	-	-	-	STa or STb or LT Positive	-	**ETEC**
Positive	-	-	-	-	-	-	Positive	**O157 H7**
Positive	Positive	-	-	-	-	-	-	**EIEC or *Shigella***
Positive	-	Positive	-	Negative	Negative	-	-	**EAEC (Enteroaggregative *E. coli*)**
Positive	-	Positive	-	Positive	and/or Positive	--	-	**EAggSTEC (Enteroaggregative Shiga-toxin producing *E. coli*)**
Positive	-	-	Positive	Negative	Negative	-	-	**EPEC (Enteropathogenic *E. coli*)**
Positive	-	-	Positive	Positive	and/or Positive	-	-	**EHEC (Enterohemorrhagic *E. coli*), also known as a type of STEC**
Positive	-	Negative	Negative	Positive	and/or Positive	-	-	**STEC (Shiga-toxin producing *E. coli*)**