MBPCR278 Hi-PCR® Acute Fever Panel Kit

Description

Acute fever illness, or acute febrile illness, is characterized by a recent onset of fever accompanied by non-specific symptoms such as headache, body rash, and muscle joint pains. The non-specific presentation of clinical symptoms poses challenge to distinguish the cause of illness based on the clinical history and physical examination alone. This may lead to delay in receiving the appropriate treatment, which may be critical for the patient. A variety of pathogens, including viral, bacterial, and parasitic agents, can cause acute fever. Common pathogens associated with acute fever include *Plasmodium* (malaria), Dengue virus, *Leptospira* (leptospirosis), Chikungunya virus, *Salmonella* (typhoid fever), Scrub typhus (caused by *Orientia tsutsugamushi*), and Zika virus. **Hi-PCR® Acute Fever Panel Kit** is an *in-vitro* multiplex probe PCR assay designed for the qualitative detection of Dengue virus, Chikungunya virus, Zika virus, *Salmonella* spp., *Leptospira* spp., *Plasmodium* spp. and *Orientia tsutsugamushi* in clinical samples. These pathogens are responsible for serious diseases prevalent in many countries, particularly those with tropical climates. Hi-PCR® Acute Fever Panel Kit detects these pathogens in a single assay with high sensitivity and specificity.

NOTE: Hi-PCR® Acute Fever Panel Kit is for *in-vitro* use only.

Intended Use: Hi-PCR® Acute Fever Panel Kit is designed for sensitive and specific detection of Dengue virus, Chikungunya virus, Zika virus, *Salmonella* spp., *Leptospira* spp., *Plasmodium* spp. and *Orientia tsutsugamushi* in clinical samples. The Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR procedures.

Principle

Hi-PCR® Acute Fever Panel kit, a multiplex Probe PCR Kit, is based on the principle of real-time PCR. The technique is used to amplify targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides having a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Acute Fever Panel kit allows simultaneous detection of Dengue virus (DENV), Chikungunya virus (CHIKV), Zika virus (ZIKV), *Salmonella* spp. (SAL), *Leptospira* spp. (LEPTO), *Plasmodium* spp. (PLAS) and *Orientia tsutsugamushi* (OT) in a two-tube assay format. Tube 1 includes primers and probes specific for Dengue virus, Chikungunya virus and Zika virus labelled with the FAM, HEX and Texas Red fluorophores respectively. Tube 2 includes primers and probes specific for *Salmonella* spp., *Leptospira* spp., *Plasmodium* spp. and *Orientia tsutsugamushi* labelled with the FAM, JOE, Texas Red and Cy5 fluorophores, respectively. In addition, tube 1 contains an endogenous internal control (IC) amplification system (probe labelled with fluorophore Cy5) to verify efficient total nucleic acid isolation from clinical samples and PCR amplification.

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WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Controls

Positive Control

Positive control (PC) is a control reaction which contain the target DNA sequence that the PCR is designed to amplify. It is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be used in every run to assess optimal performance.

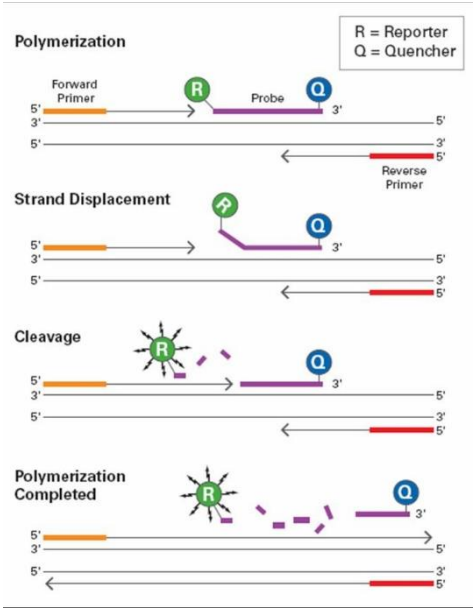
No Template Control

A No Template Control (NTC) is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA/RNA. In this reaction, nuclease free water is used as the template. It is recommended to have a minimum of one reaction of no template control per run.

Internal Control

An internal control is a sequence that is amplified alongside the target sequence from a human gene in the same reaction tube, but it is detected using a different primer-probe set. This control helps assess the quality of the clinical specimen, detect any extraction failures, identify potential PCR inhibition, and check for reagent issues.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Simultaneous detection of 7 pathogens: Dengue virus, Chikungunya virus, Zika virus, *Salmonella* spp., *Leptospira* spp., *Plasmodium* spp. and *Orientia tsutsugamushi* in a two-tube assay format
- Fast and reliable results within 110 minutes
- One-step assay i.e. reverse transcription and amplification are performed in same tube

- Includes all reagents & controls for validity of the test
- High sensitivity and specificity
- Compatible with 4-channel, 5-channel and 6-channel qPCR cyclers
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems

Sample Type: Total nucleic acids (DNA + RNA) extracted from serum of human origin

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C and -20°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 5 freeze-thaw cycles, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Exposure to light, heat or humidity may also affect the shelf life of some of the kit components and should be avoided. Degradation of specimen/ extracted DNA and RNA can also hamper the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Specimen Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred in individual safety data sheets.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R	50R	100R
DCZI Master Mix (Tube 1)	DS1760	162	324	636
SLPO Master Mix (Tube 2)	DS1761	162	324	636
DCZI Primer Probe Mix (Tube 1)	DS1762	81	162	318
SLPO Primer Probe Mix (Tube 2)	DS1763	81	162	318
Water	DS0440	486	972	1908
DCZI Positive Control (Tube 1)	DS1764	81	162	318
SLPO Positive Control (Tube 2)	DS1765	81	162	318

***** For a 30 µL PCR reaction

Materials needed but not provided

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems

Hi-PCR® Acute Fever Panel Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1	Dye 2	Dye 3	Dye 4
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/ Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	HEX	Texas Red/ROX	Cy5
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE/HEX	Texas Red/ROX	Cy5
QuantStudio™ 5 / Quant Studio™ 6 and 7 Flex Real-Time PCR Systems / QuantStudio™ Dx	Applied Biosystems	FAM	JOE/HEX/VIC	ROX	Cy5

ABI® Prism SDS 7500	Applied Biosystems	FAM	JOE/HEX/VIC	Texas Red/ROX	Cy5
QIAquant 96 & 384 5plex	QIAGEN	FAM	JOE/HEX	Texas Red/ROX	Cy5
Rotor-Gene®6000 & Q	QIAGEN	Green	Yellow	Orange	Red
LightCycler® 96 /	Roche	FAM	JOE/HEX/VIC	ROX/Texas Red	Cy5
LightCycler® 480	Roche	FAM	JOE/HEX/VIC	ROX/Texas Red	Cy5
qTOWER^3	Analytik Jena	FAM	JOE/HEX/VIC	ROX/Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufactureŕs instructions and recommendations.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the total number of specimens (including PC and NTC) to be tested (N), calculate the volume of the components to be added as **N X volume of "1R"**
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix of Tube 1 and Tube 2. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	**Product code**	**Volume for "1R" (one reaction)**	
		Tube 1	**Tube 2**
Preparation of PCR Reaction Mix			
DCZI Master Mix (Tube 1)	DS1760	6 µL	----
SLPO Master Mix (Tube 2)	DS1761	----	6 µL
DCZI Primer Probe Mix (Tube 1)	DS1762	3 µL	----
SLPO Primer Probe Mix (Tube 2)	DS1763	----	3 µL
Water	DS0440	6 µL	6 µL
Total PCR Reaction Mix	-	**15 µL**	**15 µL**
Template addition			
Template (Extracted nucleic acid)		15 µL	15 µL
Total reaction volume	**-**	**30 µL**	**30 µL**

4. Aliquot 15 µL of Tube 1 and Tube 2 PCR reaction mix into respectively labeled 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the "Nucleic acid handling" area, add 15 µL of extracted nucleic acid of test specimen into the plate/strip to respective wells.
6. For positive and no template control, template nucleic acid is replaced by Positive control 1 and 2 in tubes 1 and 2 respectively for positive control and by nuclease free water for no template control reaction in both tubes 1 and 2. Refer the following table.

Set up of **Positive controls (PC)** for the PCR run			
Components	**Product code**	**Volume for "1R" (one reaction)**	
		Tube 1	**Tube 2**
Total PCR Reaction Mix	-	15 µL	15 µL
DCZI Positive Control (Tube 1)	DS1764	15 µL	-
SLPO Positive Control (Tube 2)	DS1765	-	15 µL
Total reaction volume	-	**30 µL**	**30 µL**

Set up of **No Template Control (NTC)** for the PCR run			
Components	**Product code**	**Volume for "1R" (one reaction)**	
		Tube 1	**Tube 2**
Total PCR Reaction Mix	-	15 µL	15 µL
Water	DS0440	15 µL	15 µL
Total reaction volume	-	**30 µL**	**30 µL**

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

Sr. No	Step	Temperature	Time	Sampling	No. of cycles
1.	Reverse Transcription	50°C	15 minutes	---	1
2.	Initial denaturation	94°C	2 minutes 30 seconds	---	1
3.	Denaturation	94°C	15 seconds	---	45
4.	Annealing & Extension	58°C	60 seconds	YES	

Selection of channels:

	Target	**Channels**	**Quencher**
TUBE 1	DENV	FAM	None
	CHIKV	JOE/HEX/VIC	None
	ZIKV	Texas Red/ROX	None
	IC	Cy5	None
TUBE 2	SAL	FAM	None
	LEPTO	JOE/HEX/VIC	None
	PLAS	Texas Red/ROX	None
	OT	Cy5	None

Please select 'Passive reference dye' as 'None' wherever applicable

Threshold value set up

For the Hi-PCR® Acute Fever Panel Kit, the threshold value for some popular thermal cyclers are as follows:

Sr. No.	Real-Time PCR instrument	Threshold range
1.	HiMedia Insta Q96	600-1000[#]
2.	Applied Biosystems QuantStudio 3/5	20000-100000[#]
3.	Bio-Rad CFX-96	200-600[#]
4.	Rotor-Gene Q	Green: 0.02 Yellow: 0.045 Orange: 0.28 Red: 0.04

The threshold range value varies between different instruments depending upon the age, model and the calibration. Please contact our technical team for any queries.

[#]For analysis of Plasmodium target, threshold to be set at maximum range as per the above table. For all other targets, minimum threshold recommended should be applied.

Data Analysis

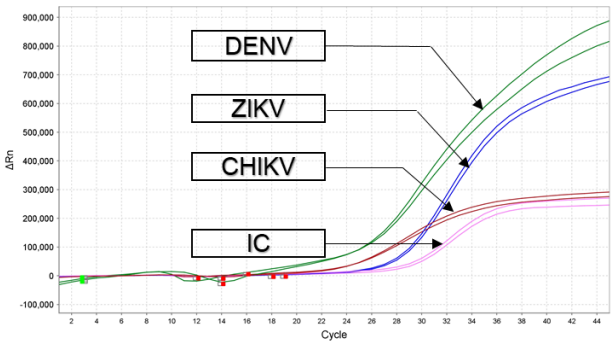
The following conditions should be met for a valid diagnostic test:

Control	Detection channel							
	TUBE 1				TUBE 2			
	FAM (DENV)	HEX (CHIKV)	Texas Red (ZIKV)	Cy5 (IC)	FAM (SAL)	HEX (LEPTO)	Texas Red (PLAS)	Cy5 (OT)
Positive Control	+	+	+	+	+	+	+	+
No template Control	-	-	-	-	-	-	-	-

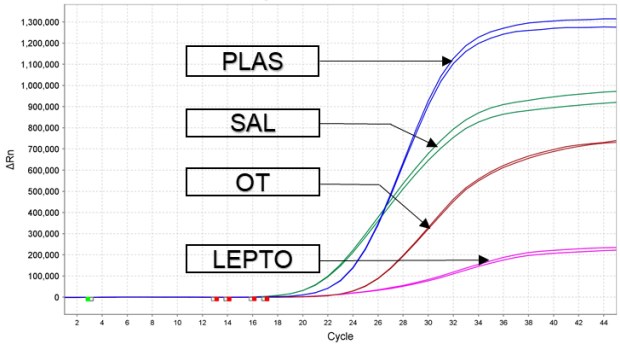
Target	Ct value	Result/Interpretation
Dengue virus (DENV)	≤ 35	Detected (+)
Chikungunya virus (CHIKV)	≤ 36	Detected (+)
Zika virus (ZIKV)	≤ 37	Detected (+)
Salmonella spp. (SAL)	≤ 37	Detected (+)
Leptospira spp. (LEPTO)	≤ 38	Detected (+)
Plasmodium spp. (PLAS)	≤ 34	Detected (+)
Orientia tsutsugamushi (OT)	≤ 36	Detected (+)

Amplification plot:

Tube 1



Tube 2



Sr. No	Tube-1	Ct value	
		PC	NTC
1	DENV	25.80	--
2	CHIKV	24.63	--
3	ZIKV	26.74	--
4	IC	29.08	--

Sr. No	Tube-2	Ct value	
		PC	NTC
1	SAL	19.41	--
2	LEPTO	23.95	--
3	PLAS	23.54	--
4	OT	23.50	--

Note: Image representing probe based Real-Time amplification of Dengue, Chikungunya, Zika, IC, Salmonella, Leptospira, Plasmodium and Orientia tsutsugamushi (Ct values provided in table are for representation) run on Applied Biosystems QuantStudio 5.

Data Interpretation:

For single pathogen infection								
Detection Channel (Target)								Result Interpretation
Tube 1				Tube 2				
FAM (DENV)	HEX (CHIKV)	Texas Red (ZIKV)	Cy5 (IC)	FAM (SAL)	HEX (LEPTO)	Texas Red (PLAS)	Cy5 (OT)	
+	-	-	+/- *	-	-	-	-	Positive for Dengue virus
-	+	-	+/- *	-	-	-	-	Positive for Chikungunya virus
-	-	+	+/- *	-	-	-	-	Positive for Zika virus
-	-	-	+/- *	+	-	-	-	Positive for Salmonella spp.
-	-	-	+/- *	-	+	-	-	Positive for Leptospira spp.
-	-	-	+/- *	-	-	+	-	Positive for Plasmodium spp.
-	-	-	+/- *	-	-	-	+	Positive for *Orientia tsutsugamushi*
-	-	-	+	-	-	-	-	Negative for Dengue virus, Chikungunya virus, Zika virus, Salmonella spp., Leptospira spp., Plasmodium spp. and *Orientia tsutsugamushi* spp. pathogens
-	-	-	-	-	-	-	-	Inconclusive test** Likely poor extraction or sample quality. PCR

								inhibition or reagent failure.

*The presence or absence of a signal in the Cy5 channel of Tube 1 is not relevant for the validity of the test run due to competition between the test template and Internal Control template.
** When an inconclusive result is obtained, repeat PCR or re-test the extracted total nucleic acid or re-extract the specimen and test with PCR.

Note: All negative findings must be correlated with clinical observations and patient history. Negative results do not exclude infections because of other variants of these viruses and should not be used as the sole basis for patient management. Co-infection of pathogens can occur and should be verified with re-test of the specimen.

Performance Evaluation

Analytical Sensitivity - Limit of Detection (LoD)
The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. The LoD assay of the Hi-PCR® Acute Fever Panel Kit was performed using 20 replicates each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems using Quantitative nucleic acids for Dengue virus, Zika virus, *Salmonella* spp., *Leptospira* spp. and *Plasmodium* spp. and synthetic nucleic acid for Chikungunya virus and *Orientia tsutsugamushi*. The detectable limit of the Hi-PCR® Acute Fever Panel Kit was determined to be 10 copies / µL for Dengue virus, 1 copy/µL for Zika virus, 1.87 copies /µL for Chikungunya virus, 1 copy/µL for *Salmonella*, 1 copy/µL for *Leptospira*, 1 copy/µL for *Plasmodium* and 5 copies/µL for *Orientia tsutsugamushi*.

Analytical Specificity

Inclusivity

The ability of the Hi-PCR® Acute Fever Panel Kit to detect a wide range of related target organisms has been assessed in the inclusivity parameter by two ways (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of related target organisms. The oligonucleotide sequences of all the targets were checked by sequence comparison against all the relevant sequences of Dengue viruses, Chikungunya viruses, Zika Viruses, *Salmonella* spp.*, Leptospira* spp.*, Plasmodium* spp. and *Orientia tsutsugamushi* available in the GenBank database.

Inclusivity was further verified by wet lab testing of the oligonucleotides (primers and probes) against commercially available controls or standards – Dengue: AMPLIRUN® DENGUE VIRUS RNA CONTROLS from Vircell Microbiologics [DENV 1 (MBC055-R), DENV2 (MBC056-R), DENV3 (MBC057-R), DENV4 (MBC058-R)], **Zika virus**: Quantitative Genomic RNA from Zika virus strain PRVABC59 (VR-1843DQ), ***Salmonella typhi***: *Salmonella typhi* (ATCC: 14028), ***Leptospira***: *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 (ATCC: 1198D-5), ***Plasmodium***: *Plasmodium falciparum* strain 3D7 (ATCC: 405D), *Plasmodium malariae* (ATCC: 3001SD).

Exclusivity / Cross-Reactivity Analysis

The ability of the Hi-PCR® Acute Fever Panel Kit to distinguish the target organisms from similar but genetically distinct non-target organisms has been assessed by (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of non-related target organisms.

The sequences of the oligonucleotides (primers and probes) in the Hi-PCR® Acute Fever Panel Kit were analyzed using BLAST (Basic Local Alignment Search Tool) against the organisms listed in the table below. These organisms were chosen if they were members of the Flavivirus family, associated with viral hemorrhagic fevers, responsible for mosquito-borne diseases, or capable of causing acute febrile illness. *In-silico* BLAST analysis showed no significant cross-reactivity with the subject sequences.

Enterovirus (taxid:12059)	*Mycobacterium tuberculosis* (taxid:1773)
Epstein Barr virus (taxid:10376)	Herpes simplex virus 1 (taxid:10298)
Herpes simplex virus 2 (taxid:10310)	Human cytomegalovirus HCMV (taxid:10359)
Burkholderia pseudomallai (taxid:28450)	*Brucella melitensis* (taxid:29459)
Measles morbillivirus (taxid:11234)	West Nile virus (taxid:11082)
Human parvovirus B19 (taxid:10798)	*Staphylococcus aureus* (taxid:1280)
Streptococcus pyogenes (taxid:1314)	Influenza B virus (taxid:11520)
Influenza A virus (H1N1) (taxid:1323429)	Influenza A virus H3N2 (taxid:41857)
SARS-CoV-2 (taxid:2697049)	*Corynebacterium diphtheriae* (taxid:1717)
Monkeypox virus (taxid:10244)	Rubella virus (taxid:11041)
Borrelia burdorferi (taxid:139)	Yersinia pestis (taxid:632)
Coxiella burnetii (taxid:777)	*Aspergillus* (taxid:5052)
Rift Valley fever virus (taxid:11588)	Human adenovirus B3 (taxid:45659)
Japanese encephalitis virus (taxid:11072)	Kyasanur Forest disease virus (taxid:33743)
St. Louis encephalitis virus (taxid:11080)	Tick-borne encephalitis virus (taxid:11084)
Yellow fever virus (taxid:11089)	*Leishmania donovani* (taxid:5661)
Agent of lymphatic filariasis (taxid:6279)	

Wet lab testing of the Hi-PCR® Acute Fever Panel Kit for potential cross-reactivity was performed using DNA/RNA from various pathogens available in the laboratory, on Applied Biosystems QuantStudio 5 PCR Systems. None of the pathogens listed in the table below exhibited any reactivity with the primers and probes of the Hi-PCR® Acute Fever Panel Kit.

Corynebacterium diphtheriae strain NCTC 13129 (ATCC: 700971D-5)	*Mycoplasma pneumoniae* strain M129-B7 (ATCC: 29342DQ)
Staphylococcus aureus subsp. *aureus* (ATCC: 43300DQ)	*Candida albicans* strain SC5314 (ATCC: MYA-2876DQ)
Borrelia burgdorferi (ATCC: VR-106DQ)	Rubella virus (ATCC: VR-315DQ)
Influenza B virus (ATCC: VR-1804DQ)	Human parainfluenza virus 1 strain C35 (ATCC: VR-94DQ)
Betacoronavirus 1 strain OC43 (ATCC: 1558DQ)	Human herpesvirus 1 (ATCC: 539DQ)
Human coronavirus NL63 RNA (ATCC: VR-3263SD)	Human herpesvirus 2 (ATCC: 540DQ)
Influenza A virus (H3N2) strain A/ Wisconsin/15/2009 (ATCC: VR-1882DQ)	Human herpesvirus 5; HCMV Strain AD-169 (ATCC: 538DQ)
Human coronavirus 229E (ATCC: VR-740DQ)	Mumps virus strain Enders (ATCC: VR-106DQ)
Human adenovirus 1 strain Adenoid 71 (ATCC: VR-1DQ)	Measles virus strain Edmonston (ATCC: VR-24D)

Evaluation

Each lot of Hi-PCR® Acute Fever Panel Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Acute Fever Panel Kit has been functionally tested in amplification assay.

General Precautions

Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

This assay must not be performed on the specimen directly. Total nucleic acids (DNA + RNA) should be extracted from human serum sample using appropriate nucleic acid extraction method.

Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen.

As with any diagnostic test, results of the Hi-PCR® Acute Fever Panel Kit need to be interpreted in consideration of all clinical and laboratory findings.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of nucleic acid using agarose gel electrophoresis.
			Use freshly prepared DNA/RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Check whether all components are added in correct volume as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure selection of appropriate fluorescence channel as detailed in the manual. Compare the PCR program to the manual.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. Use calibrated pipettes. Repeat the run.
3.	Amplification in No template control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination issues.

Safety Information

Hi-PCR® Acute Fever Panel Kit for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

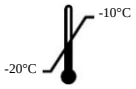
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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