# MBPCR182 Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit

## Description

Hepatitis C is a blood-borne viral infection that is caused by Hepatitis C Virus (HCV), a hepatotropic RNA virus, with a propensity to affect the liver. It is responsible for chronic liver disease and a variety of extrahepatic manifestations, hence recognized as a major public health problem worldwide. HCV is primarily transmitted via the parenteral route which includes injection drug use, blood transfusion, unsafe injection practices, and other healthcare related procedures. HCV causes acute subclinical hepatitis which gradually evolves into chronic hepatitis in about 80% of the infected cases. For long, hepatitis C remained obscure to researchers due to its clinically silent nature. Most patients with acute infection are symptom free and only a small proportion develops jaundice. Chronic HCV infection may be associated with vague, non-specific symptoms such as fatigue, joint pain, and discomfort in the right-upper quadrant of the abdomen. Patients usually become symptomatic when complications of chronic liver disease or extra-hepatic manifestations develop. Hence, early diagnosis and treatment of HCV is important which can also contribute to reducing Hepatitis transmission.

**NOTE**: HiMedia's Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is for *in vitro* use only.

## Intended Use

Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The kit is recommended for sensitive and specific detection and quantitation of HCV RNA in human plasma samples. The Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit targets 100% of the known HCV genotypes (HCV genotype 1-7).

## Product Description

Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is based on real-time PCR technology for the detection and quantitation of Hepatitis C Virus (HCV) specific RNA encompassing all major HCV genotypes (HCV genotype 1-7). The kit contains primer-probe mixture specific for detection of HCV RNA. In addition, the kit contains an internal control gene to identify the RNA extraction efficiency and to ensure successful PCR reaction. The quantitative standards (HCV quantitative standards QS1-QS4) are supplied in the kit which allow the determination of viral RNA load. The assay principle is based on hydrolysis probe chemistry which confers higher specificity and sensitivity.

## Positive control (or Quantitative standards)

This is a control reaction used to test for the presence of inhibitors in the sample or the efficiency of the polymerase chain reaction itself using a pre-dispensed nucleic acid sequence and the primer set that detects it. It is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

## Endogenous Internal control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target pathogen) but detected with a different primer (i.e. Multiplex PCR). An internal control is often



ISO 9001-2015 CERTIFIED
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

used to detect the failure of amplification in cases where the target sequence is not amplified.

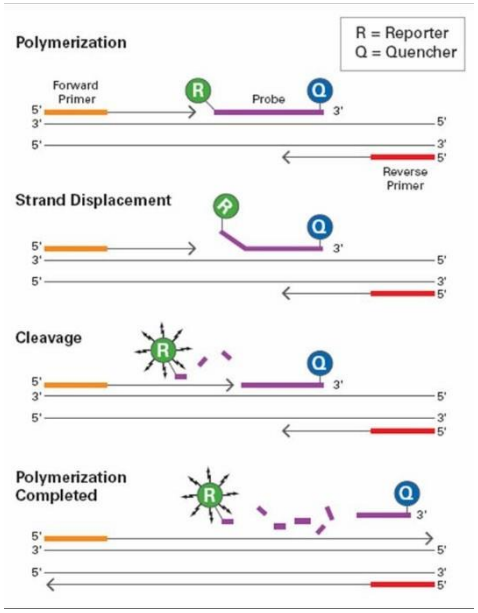
**Negative Template Control**

A negative template control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target RNA. In this reaction, Nuclease free water is used as the template. It is recommended to have a minimum of one reaction of negative control per run.

**Principle**

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted cDNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Hepatitis C Virus (HCV) Quantification Kit (Probe PCR Kit) is designed to specifically detect HCV in the FAM channel with Internal Control (IC) in ROX channel.

**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 reporter dye emission is quenched.**

**Cleavage: During each extension cycle, the DNA polymerase 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. This results in the 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**

- Detection of HCV genotypes 1 to 7.
- Highly sensitive and specific.
- Includes all reagents & controls for validity of the test.
- Includes quantitative standards for calculation of viral RNA load.
- Results within 2 hrs and 30 mins.

**Types of Specimens and storage:**

The internal validation of the Hi-PCR Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit

was performed using RNA extracted from human EDTA plasma samples. Other sample materials are not validated. Therefore, we recommend the use of EDTA plasma sample for detection of HCV. The RNA should be extracted using a standard viral RNA extraction kit. After collection, whole blood must be transported at 2-25°C and processed within 6 hours of collection. Separated plasma can be refrigerated (2–8°C) for up to 48-72 hours. Plasma specimens stored beyond these time points must be frozen at or below -20 °C. Frozen specimens should undergo no more than three freeze-thaw cycles. After extraction, store the extracted RNA samples at -20°C for short period storage and -70°C or -80°C for long period storage.

**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 to in individual safety data sheets.

**Storage and Shelf life**

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents (> 5 freeze and thaw cycle) should be avoided, as this may reduce the sensitivity of the assay. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample RNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

**Kit Contents:** The provided PCR kit contains:

| Components | Product code | Reagents provided for * (µL) | | |
|---|---|---|---|---|
| | | 25R | 50R | 100R |
| HCV Master Mix | DS1692 | 108 | 216 | 424 |
| HCV Primer-Probe Mix | DS1182 | 27 | 54 | 106 |
| Molecular Biology Grade Water | ML065 | 81 | 81 | 159 |
| HCV-QS1 ($1.5x10^6$ IU/µL) | DS0709 | 81 | 81 | 159 |
| HCV-QS2 ($1.5x10^5$ IU/µL) | DS0710 | 81 | 81 | 159 |
| HCV-QS3 ($1.5x10^4$ IU/µL) | DS0711 | 81 | 81 | 159 |
| HCV-QS4 ($1.5x10^3$ IU/µL) | DS0712 | 81 | 81 | 159 |

* For a 20 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 |
| Insta Q48® M2 Real time PCR System, 96 well block, 2 channels | LA1024 |
| TabSpin™ Microcentrifuge | LA1089/LA1090 |
| HiPer® Mini Plate Centrifuge | LA1099 |
| **Automated nucleic acid extraction system and materials** | |
| Insta NX® Mag16, Insta NX® Mag16$^{Plus}$ | LA1118, MBLA018 |

| | |
|---|---|
| Insta NX® Mag32, Insta NX® Mag32$^{Plus}$ | LA1096, MBLA019 |
| Insta NX® Mag96, Insta NX® Mag96$^{plus}$ | LA1097, MBLA026 |
| **Extraction Kits** | |
| HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification | MB582PC16 |
| HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification | MB582MPF16 |
| HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag32] | MB582MPF-32 |
| HiPurA® Prefilled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag96] | MB582MPF-96 |
| HiPurA® Viral DNA/RNA Purification Kit | MB582 |
| HiPurA® Viral RNA Purification Kit | MB615 |
| **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,100µl Max capacity 100 µL | LA1104A |
| 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, 0.1 mL, 0.2 mL; PCR Plates | PW1255/PR2/PR3/PR19 |
| Optical Sealing film | PR18 |
| 1.5 ml nuclease free Micro centrifuge tubes | PW146 |

**Kit compatibility with Real-Time PCR Systems**

Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

| Real-Time PCR system | Company | Dye 1 (HCV) | Dye 2 (IC) |
|---|---|---|---|
| Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4 | HiMedia Laboratories Pvt. Ltd. | FAM | ROX |
| QuantStudio™ 3 and 5 | Applied Biosystems | FAM | ROX |
| Applied Biosystems 7500 | Applied Biosystems | FAM | ROX |
| BioRad CFX Opus 96/CFX96 | Bio-Rad Laboratories, Inc. | FAM | ROX |
| Rotor-Gene® Q/QIAquant | QIAGEN | Green | Orange |
| Roche LightCycler® 96 | Roche | FAM | ROX |
| AriaMx | Agilent | FAM | ROX |
| Alta RT-96/48 | Athenese-Dx Private Limited | FAM | ROX |
| qTOWER$^3$ auto | Analytik Jena | FAM | ROX |

**Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufacturer´s instructions and recommendations**

**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 RNA 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.
- Clear surfaces and working areas with RNase Kil™ (ML162)

**Real time PCR Protocol:**

Prepare the PCR mix as follows in the "Master mix Preparation" area

| Components | Product code | Volume to be added for 1R (20 µL reaction volume) |
|---|---|---|
| HCV Master Mix | DS1692 | 4 µL |
| HCV Primer-Probe Mix | DS1182 | 1 µL |
| **Total Reaction Volume** | **-** | **5 µL** |

Transfer **5µL** of the above prepared PCR reaction mix in the 0.1/0.2 mL PCR reaction plate/strips/tubes, compatible to the instrument to be used.

To the **5µL** above reaction mix, add **15 µL** quantitative standard or test RNA or molecular biology grade water to make final volume **20µL**

Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film and spin.

Place the plate/strips/tubes in Real-time PCR machine and set the PCR program.

**Recommended PCR program**

| Step | Temperature | Time | Sampling | Cycles |
|---|---|---|---|---|
| 1 | 55°C | 15 minutes | --- | 1 |
| 2 | 95°C | 30 seconds | --- | 1 |
| 3 | 95°C | 10 seconds | --- | 50 |
| 4 | 55°C | 1 minute 5 seconds | Yes | |
| 5 | 72°C | 30 seconds | --- | |

**Selection of channel**

| Target | Dye | Quencher |
|---|---|---|
| HCV | FAM | None |
| IC | ROX/Texas Red | None |

**Passive Reference Dye:** Select "None"

**Data Analysis**

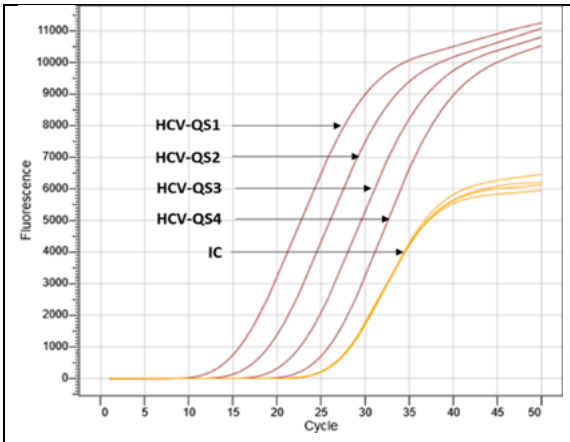
The following conditions should be met for a valid diagnostic test:

| Control | Detection channel | |
|---|---|---|
| | FAM (HCV) | ROX (Internal Control) |
| HCV-QS1 ($1.5x10^6$ IU/µL) | + | + |
| HCV-QS2 ($1.5x10^5$ IU/µL) | + | + |
| HCV-QS3 ($1.5x10^4$ IU/µL) | + | + |
| HCV-QS4 ($1.5x10^3$ IU/µL) | + | + |
| Negative Template Control | - | - |

**Standard Curve Analysis**

Interpret the values for unknown samples, only if the following conditions are met:

1. $R^2$ >0.98
2. Slope of the quantitative standards is between -3.1 to -3.7
3. PCR efficiency is between 85%-115%
4. No amplification in FAM channel of negative template control




| Sr. No. | Sample | $C_t$ |
|---|---|---|
| 1. | Negative Template Control | N/A |
| 2. | HCV-QS1 ($1.5x10^6$ IU/µL) | 15.70 |
| 3. | HCV-QS2 ($1.5x10^5$ IU/µL) | 19.45 |
| 4. | HCV-QS3 ($1.5x10^4$ IU/µL) | 22.76 |
| 5. | HCV-QS4 ($1.5x10^3$ IU/µL) | 26.08 |
| 6. | Internal Control | 28.21 |

**Image representing amplification plot of the Quantitative Standards with Ct values using HiMedia's Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit on InstaQ 96 series of instrument (Ct values provided in table are for representation only).**

**Data Interpretation:**

When all the standards fulfill the stated requirements, Interpret the results of the specimen as follows:

| Detection Channel | | Result Interpretation |
|---|---|---|
| FAM (HCV) | ROX (Internal Control) | |
| + (Ct cut off ≤ 40) | +/-* | HCV Specific RNA detected |
| - | + | HCV specific RNA is not detected. Sample does not contain detectable amounts of HCV specific RNA. |
| - | - | PCR Inhibition or reagent failure. Retest the sample. |

*Detection of the IC in the ROX channel is not required for the positive results in the FAM channel. Presence of high HCV RNA load and/or PCR inhibitors in the original sample can lead to reduced or absence of internal control signal.

**Note:**
We recommend that the test results must be interpreted by an expert. Clinical correlation along with patient history is necessary to determine patient infection status.

**Use following formula to convert concentration into IU/mL:**
The quantitation standards given in the kit are defined as IU/µL. As the initial sample volume and elution volume will be different during isolation of viral RNA, the following formula should be used to convert the concentration values determined using the standard curve into IU/mL.

$$\text{Result (IU/mL)} = \frac{\text{Concentration of sample (IU/µL) x Elution Volume (µL)}}{\text{Original Sample Volume (mL)}}$$

**Performance Characteristics**

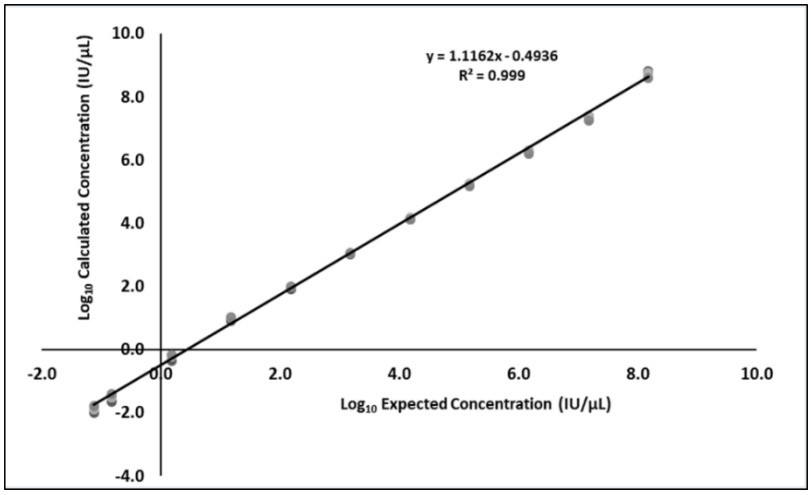
**Analytical sensitivity**
The analytical sensitivity or the Limit of Detection (LOD) of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is defined as the concentration of HCV RNA molecules that can be detected with a positivity rate of **≥ 95%**.

The analytical detection limit in consideration with nucleic acid extraction was determined using HCV spiked plasma specimens in combination with a particular extraction method. In addition, the analytical detection limit independent of extraction was determined using HCV standard of known concentration. The analytical sensitivity in consideration with nucleic acid extraction was determined using a dilution series of 6$^{th}$ WHO International Standard for Hepatitis C virus RNA for nucleic acid amplification techniques (NIBSC code: 18/184) from 2.34 x $10^4$ IU/mL to 23.4 IU/mL spiked into HCV negative EDTA plasma. Experiments were carried out in triplicate independent extractions (Sample volume: 140 µL, elution volume: 60 µL), followed by triplicate real-time PCR reactions for each extraction for three consecutive days on InstaQ 96®$^{Plus}$ real time PCR system. The analytical detection limit in consideration of the purification of Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is **≈ 43 IU/mL**.

In addition, the analytical detection limit independent of extraction was determined using HCV standard of known concentration. A dilution series of the HCV standard was set up from 1.5 x $10^8$ IU/µL to 1.5 x $10^{-2}$ IU/µL and analyzed in triplicates with the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit. The lowest dilution with limit of detection was then tested in 20 replicates. The analytical detection limit of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is **0.15 IU/µL**. The sensitivity analysis of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit was carried out on HiMedia's InstaQ 96 series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System.

**Linear Range**
The linear range in consideration of the purification of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation probe PCR Kit was determined by analyzing a logarithmic dilution series of the 6$^{th}$ WHO International Standard for hepatitis C virus RNA for nucleic acid amplification techniques (NIBSC code: 18/184) and the HiMedia's HCV quantitative standard set. Each dilution was extracted in independent triplicates (Sample volume: 140 µl, elution volume: 60 µl) and each extraction was further tested in triplicates using Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation probe PCR Kit. The linear dynamic range in consideration of the purification for the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation probe PCR Kit is 1.01 x $10^4$ IU/mL to 43 IU/mL.

The linear dynamic range of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation probe PCR Kit was determined by analyzing a dilution series of HCV from 1.5 x $10^8$ IU/µL to 1.5 x $10^{-2}$ IU/µL. The dilution series has been calibrated against the 6$^{th}$ WHO International Standard for HCV RNA. Each dilution of HCV quantitative standard for concentration ranging from 1.5 X $10^8$ IU/µL to 1.5 IU/µL was tested in triplicates; the concentration ≤ 0.15 IU/µL was tested in 9 replicates. The linear dynamic range of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation probe PCR Kit has been determined to cover concentrations from to 1.5 x $10^8$ IU/µL to 0.075 IU/µL




**Linear Range of the Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit.** The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ expected concentrations when using HCV standard.

**Cross-reactivity - Analytical Specificity**

The specificity of the kit was ensured by performing *in silico* analysis *of the* HCV primers and probes using NCBI BLAST and optimizing the stringent PCR conditions. The detectability of all the relevant HCV subtypes and genotypes was ensured by analyzing the HCV primer-probes for sequence homology to the sequences available in the HCV database (https://hcv.lanl.gov/content/index) using multiple sequence alignment tools. Wet testing analysis was performed against the pathogens available in the laboratory on InstaQ 96 series of instruments, CFX Maestro (Biorad), QuantStudio™ 5 (Thermo Fisher) for any potential cross-reactivity. Below mentioned table represents the list of pathogens analyzed for analytical specificity. None of the tested pathogen showed any reactivity to the HCV primers-probes except the HCV RNA. In addition, the specificity was validated with HCV negative plasma samples (n=25). None of the tested HCV negative samples generated positive signal with the HCV primers-probes used in the kit.

| | |
|---|---|
| Human immunodeficiency virus-1 | Human parainfluenza virus |
| Hepatitis A virus | Plasmodium falciparum |
| Hepatitis E virus | Plasmodium maleriae |
| Hepatitis B virus | Plasmodium vivax |
| Hepatitis C virus | *Klebsiella pneumoniae* |
| Dengue virus (1-4) | *Streptococcus pneumoniae* |
| Chikungunya virus | *Staphylococcus epidermidis* |
| Herpes simplex virus | *Candida albicans* |
| Human papillomavirus 16 | *Escherichia coli* |
| Human papillomavirus 18 | *Salmonella typhi* |
| Coronavirus (Covid-19) | *Mycobacterium tuberculosis* |
| Adenovirus | Blood DNA |

**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.

**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 under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

**Performance and Evaluation**

Each lot of Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Each lot of Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in RNA amplification assays.

**Troubleshooting Guide**

| Sr. No. | Problem | Possible Cause | Solution |
|---|---|---|---|
| 1. | No amplification in test and/or control wells | Degraded samples or poor-quality RNA template | Use freshly extracted, high-quality RNA. Check RNA concentration and purity (e.g., A260/280 ratio). Avoid repeated freeze-thaw cycles for stored RNA. |
| | | Missing or incorrect addition of reagents | Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps. |
| | | Incorrect thermal cycling conditions | Cross-check the PCR cycling profile with the IFU. |
| | | Expired or improperly stored reagents | Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles |
| | | Instrument malfunction | Verify that the real-time PCR instrument is functioning properly and calibrated. |
| 2. | Variability between replicates | Inconsistent pipetting or error in reaction setup | Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique. |
| | | Air bubbles in reaction mix | Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles. |
| | | Uneven mixing of reagents | Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting. |
| | | Edge effect (thermal | Avoid using outer wells in PCR plates if not |

| | | | |
|---|---|---|---|
| | | variation across plate) | temperature-uniform; use a plate seal and consistent plate layout. |
| 3. | Amplification of pathogen target in negative control | Reagent contamination | Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow. |
| | | Cross-contamination or aerosol contamination during reaction setup | Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with RNA/DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing. |
| | | Template contamination in workspace or pipettes | Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning. |
| | | Improper sealing of PCR plate/tubes | Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required. |
| 4. | No signal with positive control (Quantitative standards) or partial target amplification | Degradation of the positive control material due to improper storage or repeated freeze-thaw cycles | Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls. |
| | | Incorrect thermal cycling conditions | Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified. |
| | | Improper mixing of positive control (PC) before use | Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity. |
| 5. | Early Ct value with non-sigmoidal amplification | Instrumental noise, background fluorescence, or non-specific amplification | Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance. |
| 6. | Non-specific amplification of pathogen targets in samples and/or negative control | Improper threshold setting in the real-time PCR software | Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold as recommended in the threshold setting table. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment. |

| | | | |
|---|---|---|---|
| | | Contamination of reagents or workspace | Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes. |
| 7. | No Internal Control (IC) amplification in target-positive sample | High load of target may suppress IC amplification | If valid Ct for target is observed, it is still considered a valid result |
| 8. | High Ct values in positive samples (late amplification) | Low viral load or suboptimal sample | Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted RNA. Confirm with orthogonal test if clinically significant. |
| 9. | Inconclusive result (no amplification of target or IC) | Sample inhibition, extraction failure, or expired reagents | Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents. |
| | | Instrument malfunction | Verify that the real-time PCR instrument is functioning properly and calibrated. |
| 10. | Signal in only one replicate (of duplicate or triplicate reactions) | Pipetting error or borderline positivity | Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Borderline cases (Ct of 39-40) may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique. |
| 11. | Standard Curve does not show expected PCR parameters such as efficiency and slope | Improper Mixing of standards | Thaw and mix the standard vials properly before setting the PCR run. |
| | | 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. |
| | | Inconsistent pipetting or error in reaction setup | Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique. |
| 12. | Viral RNA detected above the linear range of the assay | High viral load sample | Dilute the RNA elute and repeat the assay. Multiply the observed IU/mL value by dilution factor. |

**Safety Information**

HiMedia's Hi-PCR® Hepatitis C Virus (HCV) Detection and Quantitation Probe PCR Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

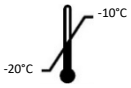
**Disposal**

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

**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 |

Identification No.: PIMBPCR182
Rev.No.:04
Date of Issue: 2025-10