IntelliPlex™ HCV Genotyping Kit User Manual

REF 82005 24 Reactions

RUO For Research Use Only

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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex HCV Genotyping Kit is a qualitative molecular assay for the differentiation of 6 major genotypes and 2 subtypes of hepatitis C virus (HCV). Based on the the πCode™ technology and PlexBio's instrument platform, the assay is designed to detect genotypes 1 to 6 and subtypes 1a and 1b of HCV from a serum- or plasma-derived sample in one single well reaction. Viral RNA needs to be purified before amplification by reverse transcription-polymerase chain reaction (RT-PCR).
The IntelliPlex HCV Genotyping Kit is not meant to be used for HCV screening of blood, serum, plasma or tissue donors, or as a diagnostic test to confirm the presence of HCV infection. The detection of genotypes 1, 2, 3, 4, 5, and 6, and subtypes 1a and 1b are intended to assist the clinician in the management of HCV infection treatments. The limit of detection is 250 IU/mL.

2. INTRODUCTION

The Hepatitis C virus (HCV) is a single-stranded RNA virus in the family Flaviviridae[1]. HCV is the major cause for post-transfusion non-A and non-B hepatitis[2]. According to the WHO about 150-180 million people are chronically infected and at risk of developing liver cirrhosis and/or cancer[3]. Hepatitis C is a blood borne illness and is the most common chronic liver disease in Europe and the United States which accounts for the majority of liver transplants performed in these regions. There is currently no vaccine for HCV. Hepatitis C virus is divided into six major genotypes and a series of subtypes. However, Genotype 1 accounts for about 60-70% of global infections. Genotypes 1-3 show a worldwide distribution, whereas Genotype 4 is mostly found in the Middle East and Egypt, Genotype 5 is almost completely limited to South Africa and Genotype 6 appears mostly in Asia[4,5,6].
Currently, treatment length, details and success rates are depending on the genotype[7,8]. It is thus important to determine the genotype and to further study the worldwide distribution and to monitor changes thereof. Detection of major genotypes 1 to 6 and subtypes 1a and 1b of the HCV from a serum- or plasma-derived sample is feasible due to an optimal primer and probe design in combination with πCode MicroDisc technology with a limit of detection of 250 IU/mL.

3. TECHNOLOGICAL PRINCIPLES

πCode MicroDisc

πCode MicroDisc is manufactured to generate up to 16,000 distinct circular image patterns for multiplexing applications. Each πCode has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. All capture agent tagged πCode are pooled, enabling capturing and detection of specific analytes in one well reaction.

Detection Principle

The procedure is based on processes listed as follows:

I. Viral RNA Purification from serum or plasma
II. RT-PCR amplification of viral nucleic acid
III. Hybridization of PCR amplicons with genotype-specific probes tagged πCode in one well reaction
IV. Incubation with SA-PE for fluorescent labelling
V. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer

4. WARNINGS AND PRECAUTIONS

- For research use only.
- This assay kit should be used by qualified laboratory personnel only.
- Do not use a kit or reagent past its expiration date.
- Sample preparation, RT-PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute; clean all equipment and surface areas regularly (e.g. The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution).
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Some reagent contains EDTA and/ or Sodium Azide in highly diluted concentration. Follow Good Laboratory Practices and Universal Precautions guidelines to avoid any risk.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition. Do not mark top of the plate.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

5. PRODUCT USE LIMITATIONS

The recommendations and procedures must be followed to prevent false results and contamination.

- The assay detects genotype 1 to 6 and subtypes 1a and 1b. The Limit of Detection is 250 IU/mL (quantification based on Roche's HCV system); for some rare subtypes the Limit of Detection may be higher.
- Limit of Detection (LoD) is based on purification of 0.2 mL plasma using QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704) and an elution volume of 25 µL.
- Genotypes with mutations/sequences different to the reference sequence[9] may not be identified correctly.
- Some rare and/ or new subtype(s) of any genotype may not be detected correctly.
- Genotype 7 will be missed.
- Infection with more than two different HCV genotypes will not be identified correctly.
- Dual infection of some rare genotype 1 and 5 or 6 sequences and other combinations with subtypes harboring a sequence different from the reference sequences may not be detected correctly.
- Dual infection with a non-similar concentration of both genotypes may be missed or not detected correctly.
- Recombination Genotypes may be missed or not detected correctly.
- Armored RNA® specific for HCV from Asuragen are not compatible with the assay (including, but not limited to, Asuragen 42101, -42004, -42006, -42008, -420010, -420012).
- Specimen collection, storage, purification and transport must be in compliance to allow efficient amplification with the RT-PCR assay.
- Mixed genotype results can be caused by mixed infection, but also recombination of HCV genotypes and/ or newly emerging subtypes/ sequences and/ or cross reactivity of πCode conjugated probes.

6. QUALITY CONTROL

The IntelliPlex HCV Genotyping Kit contains a series of internal control πCode MicroDiscs that monitor the specimen preparation, RT-PCR amplification, SA-PE incubation procedure and background noise. These controls must always meet specification and should have approximately the same intensity in each test well in the same test run. Otherwise, the test is invalid. The external controls (positive control and negative control) monitor the whole testing procedure to prevent false positive or false negative results. The test is considered invalid if any of the controls fails to meet the specified value.

7. KIT COMPONENTS

The IntelliPlex HCV Genotyping Kit contains sufficient reagents for up to 24 tests. The kit components supplied are listed as follows.

1. **HCV KIT RT-PCR Buffer**
   Ref. No.: 20009
   Quantity & Volume: 1 vial, 480 µL
   Storage: Store at -15°C to -25°C upon arrival
   Description: For RT-PCR amplification
   Contents: 6 mM MgSO4, 0.4 mM of each dNTP in buffered solution

2. **HCV KIT RT-PCR Enzyme Mix**
   Ref. No.: 20010
   Quantity & Volume: 1 vial, 72 µL
   Storage: Store at -15°C to -25°C upon arrival
   Description: For RT-PCR amplification
   Contents: RT/HotStart Taq Mix (0.1 to 0.5 Units/µL): Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase and HotStart Taq DNA Polymerase, <0.1% Synthetic oligonucleotide (46 primers), RNase Inhibitor (1 to 1.5 Units/µL)

3. **HCV KIT πCode MicroDisc**
   Ref. No.: 20015
   Quantity & Volume: 1 vial, 480 µL/vial
   Description: For RT-PCR amplicon capture
   Contents: <0.1% Synthetic oligonucleotide (34 probes), πCode MicroDisc (75 to 105 Counts/µL), 0.1% BSA, 1mM EDTA in PBS buffer.
   Preservative: <0.1% Sodium Azide

4. **HCV KIT POS Control**
   Ref. No.: 20004
   Quantity & Volume: 3 vials, air-dried
   Description: Assay positive control
   Contents: Noninfectious IVT-RNA representing HCV genotype 1b sequences derived from the 5' untranslated region (5' *UTR*), the Core coding region and Nonstructural protein 5B region (NS5B)

5. **HCV KIT NHP**
   Ref. No.: 20013
   Quantity & Volume: 1 vial, 1.5 mL/vial
   Description: Assay negative control
   Contents: Normal Human Plasma tested negative for Human Immunodeciency Virus RNA (HIV-1 RNA), Antibodies to Human Immunodeciency Virus (Anti-HIV 1/2), Antibodies to Hepatitis C Virus (HCV), Hepatitis C Virus RNA (HCV RNA), non-reactive for Hepatitis B Surface Antigen (HbsAg), and tested negative for Hepatitis B DNA. Preservative: 100 µg/mL Thimerosal, 30 µg/mL Gentamycin

6. **HCV KIT INT Control**
   Ref. No.: 20005
   Quantity & Volume: 3 vials, air-dried
   Description: Assay internal control
   Contents: Noninfectious IVT-RNA representing partial sequence of Enterobacteria phage MS2

7. **SA-PE Solution**
   Ref. No.: 20007
   Quantity & Volume: 1 bottle, 7 mL/bottle
   Description: Streptavidin-phycoerythrin for fluorescent signal acquisition
   Contents: 5 µg/mL PE-Streptavidin Conjugate, 1% BSA in buffered solution.
   Preservative: <0.1% Sodium Azide

8. **HCV KIT Hy Buffer**
   Ref. No.: 20247
   Quantity & Volume: 1 bottle, 2.4 mL/bottle
   Description: For assay hybridization
   Contents: <0.1% Sodium Azide as preservative

9. **HCV KIT 10X Wash Buffer**
   Ref. No.: 20017
   Quantity & Volume: 1 bottle, 50 mL/bottle
   Description: For πCode washing
   Contents: 1% Tween20 in buffered solution. Preservative: <0.1% Sodium Azide

10. **HCV KIT ddH2O**
    Ref. No.: 20003
    Quantity & Volume: 1 vial, 1.5 mL/vial
    Description: For reconstitution of POS and INT Control
    Contents: Nuclease-free water

NOTE:

- POS Control, NHP, INT Control and Hy Buffer stand for positive control, normal human plasma, internal control and hybridization buffer, respectively.
- Normal Human Plasma used in HCV Kit tested negative for Human Immunodeciency Virus RNA (HIV-1 RNA), Antibodies to Human Immunodeciency Virus (Anti-HIV 1/2), Antibodies to Hepatitis C Virus (HCV), Hepatitis C Virus RNA (HCV RNA), non-reactive for

Hepatitis B Surface Antigen (HbsAg), and tested negative for Hepatitis B DNA.

- AcroMetrix® HCV Mid Control (Fisher Scientific; Cat. 963002) or ZeptoMetrix Corporation NATtrol™ Human Hepatitis C Virus" (Fisher Scientific; Cat. 22-157-788) or HCV Genotype panels (e.g. AcroMetrix HCV 1-4) can be used as additional external control for the detection of HCV genotypes. AcroMetrix® Negative Control" (Fisher Scientific; Cat. 962000A) can be used as additional external negative control.
- HCV armored RNA (Asuragen) are not compatible with IntelliPlex HCV Genotyping Kit

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- 96-well plate (Greiner Bio-one; Cat. No. 655101)
- QIAamp DSP Virus Spin Kit (Qiagen, Cat. No. 61704) or QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704)
- Disposable gloves, powder-less
- Clean PCR tubes (Thin-wall 8-Strip; Fisher Scientific; Cat. 3418)
- Dedicated micropipette*
- Filter tips for micropipette*
- Vortex mixer
- Microcentrifuge
- Eppendorf® PCR Cooler or comparable (Recommended)
- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DigiPlex™ Thermocycler (PlexBio; Cat. No. 80018)
- IntelliPlex 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)
- Industrial Computer (PlexBio; Cat. No. 80002)
- DeXipher™ RU (PlexBio; Cat. No. 80050)

* Use dedicated pipettes for sample purification, sample preparation and sample hybridization. Do not share equipment between procedures. Pipettes should be accurate within 3% of stated volume. Aerosol barrier or positive displacement DNA- and RNase-free tips must be used.

9. STORAGE, STABILITY AND TRANSPORTATION

Storage

The RT-PCR Buffer and RT-PCR Enzyme Mix of the IntelliPlex HCV Genotyping Kit should be stored at -15°C to -25°C separately upon arrival.

Other kit components of the IntelliPlex HCV Genotyping Kit should be stored at 2°C to 8°C. Once opened, the reagent components are stable for 6 months or until the expiration date, whichever comes first.

Stability

Do not use the IntelliPlex HCV Genotyping Kit when it is expired. All components are guaranteed up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the IntelliPlex HCV Genotyping Kit is at 2-8°C. If the kit is broken or has components missing, please contact the PlexBio Customer Service.

10. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete installation and operation instructions (Thermocycler, IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer).

Software Installation

NOTE:

- ***For the first time assay operation, please make sure the KIT APP is installed into DeXipher.***
- ***The ENC file contains the information of kit lot no. and expiration date.***

KIT APP Installation

1. Open the HCV KIT APP from the USB drive provided and run "Installer.exe".
2. Click on "Install" from the pop up window and finish the APP installation.

Note: The KIT APP only needs to be installed for the first time running until the new version release.

ENC File Installation

1. Download the corresponding ENC File from PlexBio's website (Homepage/ Download/ ENC Files/ HCV Kit/ Corresponding Kit Lot no.)
2. Save to the computer and make sure the ENC file matches the lot no. of the assay kit.
3. Click on the button as shown below on the DeXipher homepage.

4. Click on the button as shown below to import kit.

5. Select and import the corresponding ENC file into the software.

11. SPECIMENS

Specimen Collection

The specimens could be collected as serum or plasma. EDTA as anticoagulant performs best. Heparin is not recommended as it may interfere with RT-PCR. The minimum specimen volume needed for purification processing is 200 µL.

Specimen Transportation and Storage

Serum-/ Plasma-samples: Store at or below -20°C for up to two month. Long term or improper storage may affect quality of specimen[10]. Thaw thoroughly before use; do not freeze thaw multiple times. If required, ship specimens frozen on dry ice.

Storage of Extracted RNA

Sample purified from serum or plasma specimens by using the QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704) is recommended for use with the IntelliPlex HCV Genotyping Kit.

Extracted RNA can be stored at 2°C to 8°C for up to 4 hours, or at -15°C to -25°C for up to 7 days. Long term storage is not recommended.

12. ASSAY PROCEDURE

Warning:
Read the instructions carefully and follow every step of the assay protocol correctly.

Important Handling Instructions:

Separate, dedicated areas and equipment for sample purification, sample preparation and sample hybridization must be used. Equipment (including lab coats) must not be shared between areas. All equipment and surface areas should be cleaned before and after each run (e.g. using a 0.5 – 1 % Sodium hypochlorite solution). All work should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute.

12.1 Preparation before Purification

Always include purification of at least one POS Control and one NHP sample as Negative Control.
Use of QIAamp DSP Virus Spin Kit (Qiagen, Cat. No. 61704) or QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704) with the following modifications is recommended.

No. of Samples *	Vol. Buffer AL (ml)	Vol. carrier RNA-AVE (µl)	Vol. INT control (µl)
#1	0.22	6.2	3
#8	1.76	49.3	24
#16	3.52	98.6	48
#24	5.28	147.8	72

Calculate the amount of internal control (INT Control) needed: For each sample (include NHP and POS Control), 3µL of reconstituted HCV KIT INT Control are needed; e.g. 8 samples (6 patient samples plus one POS and one NHP) require 24µL of reconstituted INT Control (= 1 vial).

Reconstitute HCV KIT INT Control:

1. Briefly centrifuge tube.
2. Add 25µL of HCV KIT ddH2O to each required HCV KIT INT Control vial (1 vial is sufficient for 8 samples).
3. Let sit for 15 minutes and ensure material is reconstituted by pipetting up and down ten times.
4. Keep vial on ice (4°C). Use within 30 minutes after reconstitution.

Reconstitute HCV KIT POS Control:

1. Briefly centrifuge tube.
2. Add 25µL of HCV KIT ddH2O to each required vial of HCV KIT POS Control.
3. Let sit for 15 minutes, and then ensure material is reconstituted by pipetting up and down ten times.
4. Keep vial on ice (4°C). Use within 30 minutes after reconstitution.

Note: POS Control and INT Control vials are provided as three single-use tubes each. After reconstitution, the POS Control and INT Control are stable without any change for up to 120 minutes.

Discard used vials. Do not reuse and freeze/ thaw any leftovers.

Buffer AL Preparation:
Prepare buffer AL as below with carrier RNA-AVE for the number of samples needed (include NHP and POS Control in the count) as indicated in Qiagen's User Manual. Add 3µL of freshly reconstituted INT Control (see table below) for each sample. Mix by inverting 20 times. Avoid formation of bubbles.

*Include POS and NEG control in count, e.g. for 6 patient sample a total of 8 samples need to be purified

Preparation of Positive Control:

1. Transfer 220µL of above prepared buffer AL (including 6.2µL RNA-AVE and 3µL INT) into a fresh tube.
2. Add 10µL of reconstituted POS Control and mix by gently pipetting up and down 20 times. Avoid formation of bubbles.
3. During purification (see below) use 200µL buffer AL including RNA-AVE and INT and **POS** (from step 2) for purification of positive control sample.

12.2 Sample Purification

Follow the instructions for QIAamp DSP Virus Spin Kit (Qiagen, Cat. No. 61704) or QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704) to do the sample purification.

Note:

1. Prepare and use buffer AL as described above.
2. **For Samples purification**, use 200µL of patient sample, 25µL QIAGEN Protease and 200µL buffer AL containing AVE-RNA and INT.
3. **For Negative Control purification**, use 200µL of HCV KIT NHP (Ref. No. 20013), 25µL QIAGEN Protease and 200µL buffer AL containing AVE-RNA and INT.
4. **For Positive Control purification**, use 200µL of HCV KIT NHP (Ref. No. 20013), 25µL QIAGEN Protease and buffer AL containing AVE-RNA and INT and **POS**.
5. Elute all samples with 25µL Buffer AVE. After addition of Buffer AVE, close lid and incubate at room temperature for 5 minutes before centrifugation.
6. Store purified samples below -20°C for up to one week (do not freeze/thaw more than once) or proceed directly to HCV RT-PCR Amplification. Always keep samples cold (4°C).

12.3 RT-PCR Amplification

1. If stored below -20°C, thaw purified samples on ice (4°C).
2. Label RT-PCR tubes with unique numbers/ names assigned. Include one tube for Positive Control and one tube for Negative Control.
3. Prepare Enzyme mix on ice (4°C).
4. Mix 20µL **HCV KIT RT-PCR Buffer** (Ref. 20009) and 3µL **HCV KIT RT-PCR Enzyme Mix** (Ref. 20010) for each reaction (= sample(s) and controls) in a RT-PCR tube.

   Note:

   - Calculate Master Mix on number of samples and controls. Always prepare a slight excess in order to ensure sufficient volume for all samples.

   - The Master Mix is stable without any change up to 120 minutes after preparation
5. Mix by vortex and briefly centrifuge all tubes before use. Once the Master Mix was prepared, add 23µl Master Mix into RT-PCR tube for each sample.
6. Keep tubes on ice (4°C; e.g. use Eppendorf® PCR Cooler or comparable).
7. Add 17µl purified sample or purified Positive or Negative Control into designated tube. Ensure proper mixing by pipetting up and down five times – avoid air bubble formation. Keep tubes on ice (4°C; e.g. use Eppendorf® PCR Cooler or comparable).
8. Spin down tubes before placing in Thermocycler.

9. Perform RT-PCR amplification with the following program:

Temp. (˚C)	Time	Cycles
50	20 min	1
95	2 min	1
95	15 sec	45
55	35 sec	
72	35 sec	
72	2 min	1
4	∞	1

**Note: Ramp rate: 5°C/sec*

12.4 DNA Hybridization and SA-PE Reaction

1. Transfer the 50mL 10X Wash Buffer to 1L Wash Buffer bottle of the IntelliPlex 1000 πCode Processor supplied and add 450 mL ddH2O to prepare 1X Wash Buffer for use.
2. Pipet the desired volume of SA-PE solution into SA-PE solution tank (V-tray). Please note that the dead volume of V-tray is **500 µL** and the minimum usage of SA-PE is **one row**.

Calculation Example:
For 3 rows reaction, the SA-PE solution needed is **400 µL x 3 rows + 500 µL = 1.7mL (at least)**.
In order to ensure the instrument has sufficient solution to dispense, pipet extra volume into the solution tank is recommended.

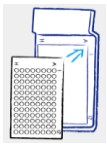
***Note:**
- *SA-PE solution should be kept in the dark.*
- ***Do not** reuse the leftover of SAPE solution and the V-tray tank.*

3. Vortex the HCV KIT πCode MicroDisc for 10 seconds, and dispenses 20 µL of πCode MicroDisc to each well (96-well plate). Vortex the πCode MicroDisc every 4 wells to ensure homogeneous suspension of the πCode MicroDisc.
4. Dispense 100 µL of HCV KIT Hy Buffer to each well.
5. Spin down the RT-PCR products.
6. Denature the RT-PCR products on the thermocycler by heating up to 95˚C for 5 minutes then cooling down to 4˚C (Ramp rate: 5°C/sec) without delay.
7. Spin down the RT-PCR products, and keep PCR products on ice (4 ˚C; e.g. in Thermocycler or use Eppendorf® PCR Cooler or comparable).
8. Add 20 µL of each freshly denatured sample to corresponding well of 96-well plate (containing Hybridization buffer and πCode MicroDisc).
9. Add 20 µL freshly denatured Positive Control sample to corresponding well.
10. Add 20 µL freshly denatured Negative Control sample to corresponding well.
11. Refer to IntelliPlex 1000 πCode Processor operation manual and follow the instructions for built-in assay program as described (Homepage/ Molecular Assay/ Select well rows/ HCV/ Confirm procedure conditions as figure shown below/ Start Running). The plate will be ready for decoding once program finished.

***Note:**
- ***Do not** open the door during the instrument operation.*

12.5 Image Decoding and Fluorescent Detection

1. Place the plate on the PlexBio 100 Fluorescent Analyzer as shown below.



2. Click on the button as shown below on the DeXipher homepage.

3. Select the well for detection and import the needed information of each detection samples. Click next step as figure shown as below.

4. Enter the “Assay Name” for the assay and click icon as shown below for image pattern decoding and fluorescent signal acquisition.

5. Export the results for data analysis or calculation or the pdf report.

13. DISCLAIMERS

Negative test result

A negative test result means the IntelliPlex HCV Genotyping Kit test was unable to determine the Genotype in the sample. It does not preclude the possibility that the specimen did in fact contain a genotype. Only samples with Genotypes matching the reference sequences[9] with concentrations above the Limit of Detection are detected; please also refer to Assay Limitations. Moreover, false negative test results may be due to experimental errors or other causes. Clinical interpretation of the results should be made in combination with all other clinical findings.

Positive test result

A positive test result means that IntelliPlex HCV Genotyping Kit test was able to determine one or multiple Genotype(s) in the sample. It does not preclude the possibility that the specimen did in fact contain a different genotype or genotypes; please also refer to Assay Limitations. False positive test results may be caused by experimental errors or other causes. Clinical interpretation of the results should be made in combination with all other clinical findings.

14. INTERPRETATION OF RESULTS

Make sure the results of external control (POS Control and NEG Control) and internal control (Internal Control, Blank and SA-PE Monitor Control) are all shown as “Pass”. Failed POS or NEG Control renders the whole assay invalid. Failed Internal Control, Blank or SA-PE Monito Control renders the affected sample invalid. Please also refer to the chapter “Troubleshooting”.

For additional information please refer to “Assay Limitations” and “Troubleshooting” section of this manual

Result Interpretation

Test Result	Report Result	Interpretation
HCV Genotype Detected	Genotype 1a Genotype 1b Genotype 1 Genotype 2 Genotype 3 Genotype 4 Genotype 5 Genotype 6	HCV Genotype detected on the specified targeted HCV region
Unrecognized Pattern	Unrecognized Pattern	1. HCV nucleic acid is detected but is with mutations/sequences different to the reference sequences 2. HCV nucleic acid is detected but is rare and/ or new subtype(s) of any genotype. 3. HCV nucleic acid is detected with mixed infection by a non-similar concentration of both genotypes or combinations of any genotypes not included in this assay
HCV Not Detected	HCV Not Detected	1. No Sample added 2. Sample titer below LoD
Invalid Assay	Invalid	1. πCode MicroDiscs Count Fail 2. SA-PE Monitor Control Fail 3. Blank Control Fail 4. Internal Control Fail

15. PERFORMANCE

Limit of Detection

Two different lots of IntelliPlex HCV Genotyping Kit were used to evaluate the Limit of Detection (LoD) for each HCV genotype. The test used one sample of each genotype from a reference HCV plasma panel. Each genotype was diluted and tested at four different concentrations, ranging from 250 to 25 IU/mL. Each prepared sample (= each dilution for each genotype) was tested for a total of 21 replicates in 3 days (= a total of 7 replicates for each day).

The Limit of Detection (LoD) for the IntelliPlex HCV Genotyping Kit was determined with 250 IU/mL (quantification based on Roche's HCV system).

Limit of Detection Estimates (IU/mL)

Lot	Genotypes						
	1a	1b	2	3	4	5	6
A	250	125	125	250	125	250	50
B	250	125	125	125	125	250	50

Serum Compatibility

The serum compatibility was evaluated by testing HCV genotypes 1a, 1b and 2 through 6 in serum as matrix with the IntelliPlex HCV Genotyping Assay. Each HCV positive sample was diluted to 250 IU/mL in Pooled Normal Human Serum. A total of 21 replicates using two different lot reagents across 3 days were tested.
All genotypes could be detected correctly at 250 IU/mL with hit rates exceeding 95%. The results demonstrate that the IntelliPlex HCV Genotyping Kit assay is compatible for serum specimen.

Serum Compatibility Analysis for HCV Genotypes

Geno - types	Conc. Level (IU/mL)	Total Number of Samples	Number of Correctly Identified Samples	Hit Rate Percentage
1a	250	42	41	97.6 **%**
1b	250	42	41	97.6 **%**
2	250	42	42	100 **%**
3	250	42	41	97.6 **%**
4	250	42	40	95.2 **%**
5	250	42	40	95.2 **%**
6	250	42	42	100 **%**

Accuracy

The accuracy of IntelliPlex HCV Genotyping Kit was tested by using a reference HCV panel reflecting 6 x Genotype 1 (3 x 1a; 3 x 1b), 5 x Genotype 2, 4 x Genotype 3, 5 x Genotype 4, 4 x Genotype 5 and 4 x Genotype 6. The genotypes of all samples were determined using the IntelliPlex HCV Genotyping Kit on two different days.

Genotypes of the reference panel were confirmed using Innogenetics INNO-LiPA HCV assay, Quest Diagnostics Hepatitis C Viral RNA Genotype LiPA assay, Siemens TRUGENE HCV Genotyping assay or PCR/Sequencing methods.

The percentage of correctly identified samples for the above listed assay condition was 100%.

Accuracy Analysis for HCV Genotypes

Geno-types	Total Number of Tested Reference Genotypes (Day 1/ Day 2)	Number of Correctly Identified Genotypes (Day 1/ Day 2)	Percent Correctly Identified
1a	3 / 3	3 / 3	100 %
1b	3 / 3	3 / 3	100 %
2	5 / 5	5 / 5	100 %
3	4 / 4	4 / 4	100 %
4	5 / 5	5 / 5	100 %
5	4 / 4	4 / 4	100 %
6	4 / 4	4 / 4	100 %

Specificity with HCV-Negative Specimens

The performance of IntelliPlex HCV Genotyping Kit was also tested by purifying 30 individual patient samples that had previously been confirmed negative for HCV. 4 replicates of each HCV negative samples were tested using 2 different lots (duplicate for each lot in one run). The observed percentage of "No HCV Signal" for negative specimens was 100%.

Reproducibility

The reproducibility of the IntelliPlex HCV Genotyping Kit was evaluated using 7 different genotypes (1a, 1b and 2 through 6) at two different concentrations (750 and 375 IU/mL). The study was conducted at three different testing sites. At each site, two different reagent lots were utilized in 5 non-consecutive days, resulting in a total of 10 runs. Each run included two replicates of each genotype at both concentrations. A total of three different reagent lots were used during this study.

The correctly identified percentage of the IntelliPlex HCV Genotyping Kit was 99.0% (832/840) covering all genotypes including 1a, 1b, and 2 through 6.

Reproducibility Study for all Sites and Lots Combined

Geno-types	Conc. Level (IU/mL)	Total Number of Tested	Number of Eligible Results	Percent Correctly Identified
1a	750	60	60	100 %
	375	60	59	98.3 %
1b	750	60	60	100 %
	375	60	59	98.3 %
2	750	60	59	98.3 %
	375	60	58	96.7 %
3	750	60	60	100 %
	375	60	60	100 %
4	750	60	59	98.3 %
	375	60	60	100 %
5	750	60	60	100 %
	375	60	59	98.3 %
6	750	60	60	100 %
	375	60	59	98.3 %
Total		840	832	99.0 %

Interference

HCV Interference test assess the impact of different drugs and compounds potentially presented in patient samples on IntelliPlex HCV Genotyping assay. HCV negative samples and two concentration level of HCV genotype 1b samples ($2.5x10^4$ and $7.5x10^2$ IU/mL) were spiked with 9 different groups of drugs/compounds and tested (listed in table below). All drugs/compounds were used at a concentration at least 5 times higher than the reported peak plasma level.

Each group (1-9) and sample type (HCV low and high titer and HCV negative) were tested in four experiments, resulting in a total of 108 tests. The percentage of correctly identified samples was 100%, demonstrating that none of the tested drugs/compounds interferes with the IntelliPlex HCV Genotyping Kit.

Potentially Interfering Substance Tested

Groups	Drugs/Compounds
1	Nevirapine, Efavirenz, Amprenavir, Saquinavir mesylate/Invirase, Nelfinavir mesylate hydrate
2	Zidovudine, Lamivudine, 2',3´-Didehydro -3´-deoxythymidine/Stavudine-D4, Abacavir sulfate
3	Enfuvirtide acetate salt, Adefovir
4	Interferon α 2A human, Interferon α 2B human, Ribavirin
5	Acyclovir, Azithromycin, Clarithromycin
6	Valganciclovir hydrochloride, Ciprofloxacin, Hemoglobin human
7	BILIRUBIN, Lipid standards: triglyceride mixtures
8	DMSO control
9	Nuclease-free water control

Cross-Reactivity

The Cross Reactivity assesses the impact of non-HCV viral and bacterial infections on the IntelliPlex HCV Genotyping assay. HCV negative samples and HCV genotype 1b samples at 750 IU/mL were tested alone or in combination with high titers of inactivated non-HCV virus or bacteria. For some viruses and bacteria, high concentration of purified non-HCV viral and bacterial nucleic acid was used in the tests.

Each combination was tested four times, resulting in a total of 176 tests. The percentage of correctly identified samples was 100%, demonstrating no cross-reactivity of non-HCV infections with the IntelliPlex HCV Genotyping Kit.

List of Inactivated Virus or Bacteria Tested

Human immunodeficiency virus 1 (HIV-1)
Human T-lymphotropic virus I (HTLV-I)
Human T-lymphotropic virus II (HTLV-II)
Hepatitis B virus (HBV)
Epstein-Barr virus (EBV)
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Human herpesvirus 6 (HHV-6)
Human herpesvirus 8 (HHV-8)
Cytomegalovirus (CMV)
Varicella-zoster virus (VZV)
West Nile Virus (WNV)
BK human polyomavirus
Neisseria gonorrhoeae
Chlamydia trachomatis

List of Purified Viral or Bacterial Nucleic Acid Tested
Human papilloma virus 16 (HPV-16)
Human papilloma virus 18 (HPV-18)
Dengue Virus (DV)
Staphylococcus aureus
Human genomic DNA

Mixed Infection

The IntelliPlex HCV Genotyping Kit is designed to detect genotypes in samples with mixed infections. A panel consisting of mixed HCV genotype specimens, representing all possible combinations of HCV genotypes 1a, 1b, and 2 through 6, were prepared for testing. Each distinct genotype combination was tested at 1 x 10^5:1 x 10^5 IU/mL, 1 x 10^5:250 IU/mL and 250:250 IU/mL. A total of 84 samples were tested.

The IntelliPlex HCV Genotyping Kit detected both genotypes correctly for samples with equal concentrations of both genotypes (100 %). In mixed infections of unequal concentration, the kit detected in most cases only the major genotype at the higher concentration. In few cases both genotypes were detected correctly or an unrecognized pattern was obtained.

Cross-Contamination/ Carryover

The test evaluates cross-contamination/ carryover risk during the assay procedure which may lead to false positive or wrong results.

One experiment was performed on five days. Each experiment utilized ten HCV positive and ten HCV negative samples. Positive and Negative samples alternated. A total of 100 samples were tested.

The results demonstrated that there was no cross-contamination/ carryover during the assay procedure.

16. SYMBOLS

Symbol	Explanation	Symbol	Explanation
RUO	For research use only	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture

17. REFERENCES

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18. TROUBLESHOOTING

The troubleshooting guide listed below addresses possible problem causes and solutions provided during assay procedures.

Problem	Possible Cause	Recommendations
No Valid Assay Assigned	1. No plate inserted. 2. Plate inserted in wrong orientation. 3. No HCV APP installed. 4. No ENC file imported. 5. Two or more lots of reagent used.	1. Confirm plate is inserted and repeat reading. 2. Confirm orientation of plate and repeat reading. 3. Install HCV APP and repeat reading. 4. Import ENC file and repeat reading. 5. One reagent lot used at the one time.
Positive Control Fail / Negative Control Fail	Positive Control must be positive for Genotype 1b. Negative Control must be HCV negative. 1. No POS Control, INT Control or NEG Control added. POS Control or INT Control not proper reconstituted. 2. RNase contamination. 3. Assay did not work. 4. A different Genotype than 1b in PC well has been detected. Cross contamination between samples. 5. Wrong PC/NC wells chose.	1. Ensure proper reconstitution of POS Control and INT Control. Do not freeze/ thaw reconstituted POS Control and INT Control. Ensure preparing and using AL buffer with POS Control and INT Control as described. 2. Ensure all the RNA operating procedures are followed correctly. Ensure work environment is free of RNase. 3. Make sure all the assay procedures are followed correctly. 4. Ensure no contamination between samples. Clean all surfaces and equipment. Keep sample purification, preparation and hybridization areas and equipment separated. 5. Choose the correct PC/NC wells and repeat reading.
πCode MicroDiscs Count Fail	DeXipher is unable to detect sufficient numbers for all the πCode MicroDiscs to allow HCV Genotype determination: 1. πCode MicroDiscs are not proper dispensed. 2. Not enough πCode MicroDiscs added to well. 3. Loss of πCode MicroDiscs during assay performance. 4. Instruments error or malfunction.	1. Re-dispense πCode MicroDiscs and repeat reading. 2. Ensure HCV πCode MicroDiscs is mixed well before adding to well and ensure proper amount is added. Repeat test. 3. Ensure following the protocol step-by-step. Repeat test. 4. Contact PlexBio Customer Service.
SA-PE Monitor Control Fail	Performance of SA-PE is assessed by the SAPE Monitor Control. 1. No SA-PE was added. 2. SA-PE solution inactivation. 3. Incorrect tested rows of microplate selected for SA-PE solution dispensing.	1. Make sure all the assay procedures are followed correctly. Repeat test. 2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date. 3. Repeat assay and make sure rows selected correctly.

Blank Control Fail	"Background" is determined by measuring MFI of an internal control that should not give a signal. 1. Residues of SA-PE solution in wells after hybridization. 2. PlexBio 100 Fluorescent Analyzer is not calibrated. 3. Markings on plates.	 1. Ensure all buffers (Wash buffer and ddH2O) on IntelliPlex 1000 Processorare sufficient for washing procedures. 2. Perform calibration on PlexBio 100 Fluorescent Analyzer. 3. Do not make any marking of plate. Some markers give fluorescence background.
Internal Control Fail	Internal Control monitors all steps in the procedure and must be positive. 1. No Internal Control added or Internal Control not proper reconstituted. 2. Purification failed. RT-PCR inhibitor remained. 3. RT-PCR not performed correctly. 4. RNase contamination. 5. Hybridization did not work.	 1. Ensure proper reconstitution of Internal Control. Do not freeze/ thaw reconstituted Internal Control. Ensure preparing and using AL buffer with Internal Control as described. 2. Follow purification instruction carefully. Ensure required temperature ranges are complied. Ensure required centrifugation needs are complied. Ensure work environment is free of RNAse. Ensure complete removal of ethanol. 3. Make sure all the RT-PCR procedures are followed correctly. Do not to use expired material. Ensure storage conditions are correct. 4. Ensure all the RNA operating procedures are followed correctly. Ensure work environment is free of RNAse. 5. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured.
HCV Signal Fail	1. No Sample added. 2. Sample titer below LoD. 3. Unknown emerging mutation in HCV sequence.	1. Repeat Assay and ensure adding sample. 2. Titer may differ between different. quantitative HCV molecular assays. The LoD of IntelliPlex HCV Genotyping Kit is 250 IU/mL based on Roche Cobas system. 3. Perform sequence analysis of sample to determine genotypes.
Unrecognized Pattern	"Unrecognized Pattern" will be displayed if all controls performed correctly but the combination of positive/ negative genotyping πCode MicroDiscs does not correlate to a unique pattern for any of the 6 Genotypes or combinations.	Perform sequence analysis of sample to determine genotypes.