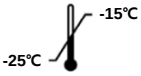
USER'S GUIDE

CE 0197

Innovation • Value • Discovery

AccuPower®
HCV Quantitative RT-PCR Kit



REF **HCV-1211**

IVD Quantitative test kit
Hepatitis C virus RNA

EC REP MT Promedt Consulting GmbH Altenhofstr. 80
D-66386 St. Ingbert. Germany. Tel +49 6894-58 10 20

BIONEER
Innovation • Value • Discovery

AccuPower® HCV Quantitative RT-PCR Kit

User's Guide

Version No.: 3.8 (2020-10-08)

Please read all the information in booklet before using the unit

Bioneer Corporation
8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon
34302, Republic of Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8688
Email: sales@bioneer.co.kr
www.bioneer.com

Safety warning and Precaution

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Please read the User's Guide and check the integrity of all tubes, tips and other materials supplied with this kit prior to use.

Before, during and after use of this kit as described in this User's Guide, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/ government in which this product is being used. Adhere to general clinical laboratory safety procedures during the experiment.

Warranty and Liability

All BIONEER products are manufactured and tested under strict quality control protocols. BIONEER guarantees the quality of all directly manufactured products until the expiration date printed on the label. If any issues are discovered relating to compromise in product quality, immediately contact BIONEER's Customer Service Center (order@bioneer.com).

BIONEER does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the appropriate and applicable User's Guide. BIONEER assumes liability under the condition that the user discloses all information related to the problem to BIONEER in written form within 30 days of occurrence.

Legal Disclaimer

Some applications that may be performed with this kit may infringe upon existing patents in certain countries. The purchase of this kit does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on country and application. BIONEER does not condone nor recommend the unlicensed use of a patented application.

The use of the kit is only for qualified and well-trained users in handling of clinical specimens and molecular biological experiments. After testing, all wastes should be processed with the fulfillment of the regulation of the country.

Trademark

Copyright

Notice

Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice. All information provided here is subject to change without notice.

Contents

1. INTENDED USE

AccuPower® HCV Quantitative RT-PCR Kit is an in vitro diagnostic kit designed for the quantification of HCV (Hepatitis C Virus) RNA in human EDTA-plasma samples or in human serum through real-time polymerase chain reaction (PCR) using *ExiStation*™ Universal MDx system. *AccuPower*® HCV Quantitative RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for monitoring of patient`s prognosis or antiretroviral therapy by measuring HCV genotype 1-6 within range of 8.00 to 1.30 Log_{10} IU/㎖ in EDTA-plasma and Human serum. This kit is intended for HCV viral load test and not for a screening test for HCV infection in clinical samples including blood and blood products.

2. INTRODUCTION

Hepatitis C virus (HCV) is positive sense single strand RNA virus in the family *Flaviviridae*, it is spherical and lipid-enveloped. Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide[1)]. HCV was discovered in the late 1989 and HCV is usually transmitted by blood and blood products or individuals used intravenous drugs. In addition, infants born to an infected mother or having sexual intercourse with an infected partner are highest risk factors.[2)] Seven genotypes (6 major genotypes and 1 minor genotype) of HCV have been identified to date. Genotypes 1-3 have a worldwide distribution. Genotypes 4 and 5 are found principally in Africa, and genotype 6 is distributed in Asia and genotype 7 was found in democratic Republic of Congo.[3)4)]

Detection of antibodies to HCV (anti-HCV) is recommended to first line diagnostic test for HCV infection. If anti-HCV antibodies are detected, sensitive molecular method (i.e HCV NAT assay) is strongly recommended to detect a HCV Ribonucleic acid (RNA) before the start of antiviral therapy. Quantitation of HCV RNA for measuring viral loads and for on-treatment viral loads are well established. Specific drug is recommended to the patient according to HCV genotype. For example, IFN-α is recommended for HCV genotype 1 and ribavirin for HCV genotype 2-3 [2)5)].

AccuPower® HCV Quantitative RT-PCR Kit allow detection of HCV genotype 1-6 which is utilized from nucleic acid extraction to qPCR using Bioneer`s own Mdx system(*ExiStation*™ System). Bioneer`s own Mdx system is utilized from nucleic acid extraction to qPCR. This kit is intended for HCV viral load test and not for a screening test for HCV infection in clinical samples including blood and blood products.

3. FEATURES AND PRINCIPLE OF THE TEST

Real-time PCR involves the selective amplification of a target sequence (5`-UTR) while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. PCR Reverse transcriptase from the initial RNA promotes the synthesis of the cDNA. After the synthesis, PCR amplification by DNA Polymerase proceeds. The specificity is provided by a pair of specific primers, along with a hydrolysis probe which is also sequence specific. Monitoring amplified product is conducted by labeling the hydrolysis probe with a matched pair of fluorescent dyes (5'-Fluorescent reporter; 3'- Quencher). Due to fluorescence resonance energy transfer (FRET), an intact probe will not emit light. However, upon cleavage by the 5' - 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule will emit a specific wavelength of light within the visible spectrum when cleaved after binding to the amplicon.

The kit was designed to maximize reproducibility and ease-of-use by vacuum-drying all reagents for PCR including primers, probes, DNA polymerase, dNTPs and salts by using our proprietary stabilization technology to preserve the full activity of the mixed reagents. The primer-probe set was selected from a pool of primer- probe combinations designed by bioinformatics algorithms to achieve maximized amplification efficiency and to match the thermal cycler program with all of our other *AccuPower*® Diagnostic Kits. So that, this product could be run simultaneously with other kits from *AccuPower*® Diagnostic Kit series.

4. CONTENTS AND RELATED INSTRUMENTS

4.1 Contents of the Kit

No.	Reagent	Unit	Components	Safety symbol and warning	Quantity	Function
①	HCV Premixed qPCR tubes	8-well strip X 12 ea (96 tests) (in aluminum foil bag)	Tris buffer, potassium chloride, magnesium chloride, Taq polymerase, dNTP, Reverse Transcriptase, primer/probe for HCV and IPC detection, DL-Dithiothreitol, 0.01% Tween 20, RNase Inhibitor		1 pack	NA amplification
②	HCV SPC[a] (S1) (4,000 copies/㎖)	1300 ㎕ / tube (Green 2㎖ screw tube)	Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin	-	1 tube	Calibration
	HCV SPC (S2) (40,000 copies/㎖)				1 tube	
	HCV SPC (S3) (400,000 copies/㎖)				1 tube	
	HCV SPC (S4) (4,000,000 copies/㎖)				1 tube	
	HCV SPC (S5) (40,000,000 copies/㎖)				1 tube	
③	HCV LPC[b] (4,000 copies/㎖)	1300 ㎕ / tube (Blue 2㎖ screw tube)	Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin	-	3 tubes	Positive Control
	HCV HPC[c] (400,000 copies/㎖)	1300 ㎕ / tube (Red 2㎖ screw tube)	Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin		3 tubes	Positive Control
④	HCV NTC[d]	1300 ㎕ / tube (Clear 2 ㎖ screw tube)	DEPC-DW, 0.05% Acetylated Bovine serum albumin		3 tubes	Non Template Control
⑤	SL buffer	1300 ㎕ / tube (Clear 2 ㎖ screw tube)	DEPC-DW, 0.05% Acetylated Bovine serum albumin		2 tubes	Sample dilution
⑥	Optical sealing film	-	-		1 ea	Sealing of premix well
⑦	Quick Manual	-	-		1 ea	
⑧	User Guide	-	-		1 ea	Provide by e-mail or directly

a : Standard Positive Control b : Low Positive Control c : High Positive Control d : Non Template Control

4.2 Related Instruments

This kit is optimized for use with BIONEER's *ExiStation*™ Universal Molecular Diagnostic System (A-2200, A-2200-N, A-2400, A-2410). For detailed operating instructions of each device, please refer to the instrument *User's Guide*.

5. STORAGE CONDITION AND SHELF LIFE

The *AccuPower*® HCV Quantitative RT-PCR Kit should be stored at -25 ~ -15℃ away from UV/sunlight. The kit is guaranteed stable until the expiration date (12 months) printed on the label. Repeated thawing and freezing of HCV premixed qPCR tube, the SPCs (HCV SPC (S1)-(S5)) and PCs (HPC/LPC) should be avoided, as this may reduce assay performance. If intermittent use of the kit and component (HCV premixed qPCR tube, the SPCs and PCs) is expected, HCV premixed qPCR tube are stable for up to 10 freeze/thaw cycles and SPCs (HCV SPC (S1)-(S5))/ PCs (HPC/LPC) are stable for up to 3 freeze/thaw cycles.

6. REQUIRED MATERIALS AND EQUIPMENT

System	Instrument	Reagent (Extraction)
***ExiStation*™ (A-2200)**	-*ExiPrep*™16 Dx (Cat. No. A-5050) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)	- *ExiPrep*™ Dx Viral RNA Kit (K-4473) -*ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471) -Sample Loading Tube_RNA IPC (Cat. No. KA-3011)
	- *Existation* manager software(Version 1.02. XX)	
***ExiStation* ™ (A-2200-N)**	-*ExiPrep*™16 Dx (Cat. No. A-5050) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1)	- *ExiPrep*™ Dx Viral RNA Kit (K-4473) -*ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471) -Sample Loading Tube_RNA IPC (Cat. No. KA-3011)
	- *Existation* manager software(Version 4.02. XX)	
***ExiStation* ™ 48 (A-2400)** ***ExiStation* ™ 48A (A-2410)**	- *ExiPrep*™48 Dx (Cat. No. A-5150) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1) -*ExiLT* (Cat.No. A-7100)	- *ExiPrep*™48 Viral DNA/RNA Kit (K-4571) - *ExiPrep*™48 Viral RNA Kit (K-4573) -*Exiprep*™48 Sample Loading Tube_RNA IPC (KA-4502)
	- *Exiprep* 48 software(Version 1.0.X.X)	
Etc	- *ExiSpin*™ (Cat.No. A-7040)	N/A
General lab- equipment and disposables	• Disposable powder-free gloves • Pipette set appropriate volume (1,000 ㎕, 200 ㎕, 20 ㎕ pipette) • Sterilized pipette tips with filters(1,000 ㎕, 200 ㎕, 20 ㎕ tips with filters) • 1.5 ㎖ or 15 ㎖ conical tubes	

7. GENERAL PRECAUTIONS

- Real-Time PCR with this kit should be performed using *Exicycler*™ 96 Real-Time Quantitative thermal block.
- Please read this User's Guide before use.
- All patient`s specimens should be handled as infectious material.
- Always wear gloves, laboratory coat and a mask when handling specimen or agents.
- Change gloves after contact with potential contaminations, e.g. specimens, eluents, etc.
- Wash hands thoroughly after handling specimen and reagents and taking off the gloves.
- Do not pipette by mouth.
- Do not eat, drink or smoke in dedicated working area.
- DO NOT re-use opened reagents and do not mix reagents from different production lots.
- DO NOT change the protocol as described in this User's Guide.
- Always use sterile, disposable filtered-pipette tips.
- Clinical samples and their derivatives should be stored in a separate location/ freezer from where the rest of the kit components are stored.
- DO NOT freeze whole blood or any samples stored in primary tube.
- All kit components should be allowed to slowly thaw for at least 10 minutes before initiating an experiment.
- Briefly vortex and spin-down all kit components after thawing to ensure optimum results.
- All SPC or PCs should be added in a physically separate location from where the premix is reconstituted.
- Take caution, when using a scissor or cutter.
- Clean and disinfect spilled specimens and/or dedicated working area with 0.5% sodium hypochlorite in distilled or deionized water (1:10 dilution of liquid household bleach) and should be thoroughly rinsed with 70% ethanol or distilled water.
- DISCARD A WASTE (liquid, plastic ware or biological waste) according to local safety regulation or internal laboratory procedures.

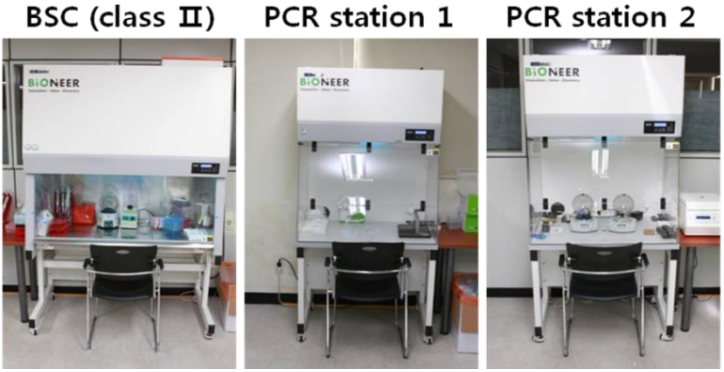
8. PROTOCOL

8.1 Laboratory equipment and environment

We recommend that several precautionary measures be taken for the safety of user and laboratory, and also for the prevention of laboratory environmental contamination.

When handling clinical samples, all related works (i.e. de-capping, pipetting, capping of clinical samples and containers) **should be conducted within a negative pressure biosafety cabinet (class Ⅱ or Ⅲ).** Negative pressure biosafety cabinet sends air from the laboratory space outside. In other words, air flows inward. This airflow prevents dangerous substances from contaminating the laboratory environment.

When opening sterilized containers such as Buffer Cartridges (*ExiPrep*™ Dx prep kit series), the work should be conducted in a positive pressure environment to prevent environmental contaminants from entering and fouling the sterile supplies. Positive pressure biosafety cabinet is a workspace where filtered air flows outward, thus keeping a clean environment within the workspace.



Handling clinical specimens | Preparation of nucleic acid preparation kit contents | After the nucleic acid preparation, sealing and vortexing the PCR tubes.

Fig. 1 Biosafety Cabinet (BSC)

8.2 Specimen

All samples should be treated as potential biohazards. For the best results, we recommend RNA extracted from human EDTA-plasma or human serum samples.

8.2.1 Specimen Collection

The *AccuPower*® HCV Quantitative RT-PCR Kit is optimized for RNA extracted from human EDTA-plasma sample or from human serum. For EDTA-plasma collection, standard specimen collection tubes such as disposable tubes containing EDTA as anticoagulant can be used. All samples should be kept in preservative-free containers.

8.2.2 Specimen Transport

All samples should be transported in a shatterproof transport container to prevent potential infection from sample leakage. Samples should be transported according to local/national guidelines regarding biohazard transportation. Whole blood collected in tubes should be stored and/or transported within 12 hours at 2°C to 25°C.

8.2.3 Specimen Storage

The isolated human EDTA-plasma or human serum can be stored up to 7 days at 2~8°C or up to 4 weeks at -80 to -15°C. Plasma or human serum samples are stable for up to 3 freeze/thaw cycles when stored frozen at -25 to-15°C.

8.2.4 Interfering Substances

Clinical samples may contain a variety of PCR inhibitors. For efficient PCR, such inhibitors must be removed during the RNA extraction and purification process.

8.3 Work Flow

The *AccuPower*® HCV Quantitative RT-PCR Kit is designed for use with *ExiStation*™ Universal Molecular Diagnostic System.

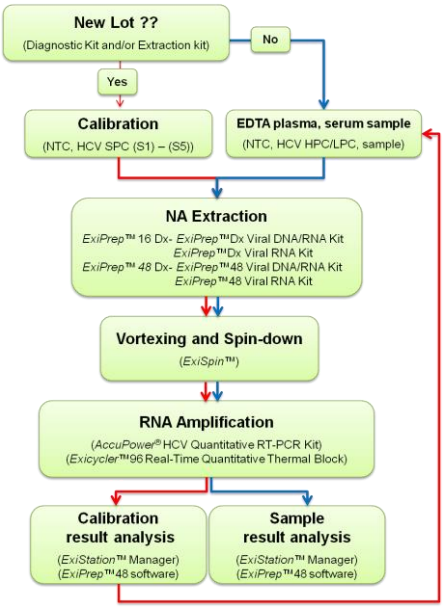


Fig. 2 Work flow

When using the kit in accordance with *ExiStation*™, both nucleic acid extraction and PCR should be conducted according to the protocol described in this User's Guide. The PCR can be performed without additional steps for preparing PCR mixture when *ExiStation*™ Universal Molecular Diagnostic System is used. After completing PCR, the data can be automatically analyzed through *ExiStation*™ Manager software. For further instructions, please refer to this User's Guide (8.4~8.5 Procedure).

8.4 Experimental procedure Ⅰ (*ExiStation*™ system)

Part 1. Assigning test using *ExiStation*™ Manager program

*** The *ExiStation*™ Universal Molecular Diagnostic System utilizes automated nucleic acid extraction on the *ExiPrep*™16 Dx instrument with *ExiPrep*™ Viral DNA/RNA Kit (K-4471) or *ExiPrep*™ Viral RNA Kit (K-4473). For further information on the extraction, refer to the User's Guide.**

1) Turn on the computer, which is pre-installed with *ExiStation*™ Manager Software.

2) Execute the *ExiStation*™ Manager Software by clicking the icon located on the desktop.

Fig. 3 *ExiStation*™ Manager Software icon

3) Turn on the *ExiPrep*™16 Dx (A-5050) by pressing the main power button located at the front of the instrument. Press the 'STARTING' image displayed on the LCD to initiate instrument startup.

Fig. 4 Starting button and main power button of *ExiPrep*™16 Dx

4) Press the 'MISC SET' button on the LCD screen (or the 'Load' button on the software).

Fig. 5 LCD screen of *ExiPrep*™16 Dx and Load button of *ExiStation*™ Manager Software

5) Attach the filter paper onto the Contamination Shield. Attach the prepared Contamination

Shield then the Tip Protector in the instrument. Press the 'Misc Set' button again.

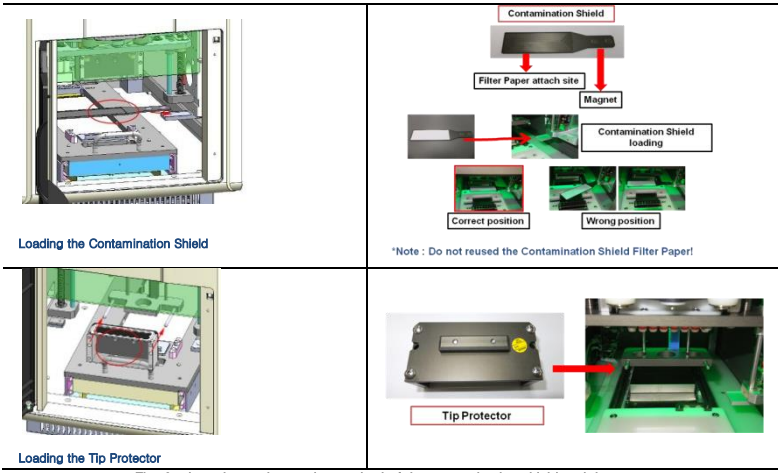


Fig. 6 Location and mounting method of the contamination shield and tip protector

6) Shut the instrument door. And Press the 'UV lamp' button on the LCD screen.

Fig. 7 LCD screen of *ExiPrep*™ 16 Dx

7) *ExiStation*™ Manager Software has six distinct parts.

Prep – control nucleic acid extraction (*ExiPrep*™16 Dx instrument),
Assign PCR – transfer sample information from 'Prep' to 'PCR' (*Exicycler*™ 96) and assign for PCR run
PCR – show real-time amplification conditions (*Exicycler*™ 96)
Result – when PCR is complete, present result, experiment and sample information
Configuration – software set-up information (accessible only by manufacturer)
Version – present software version

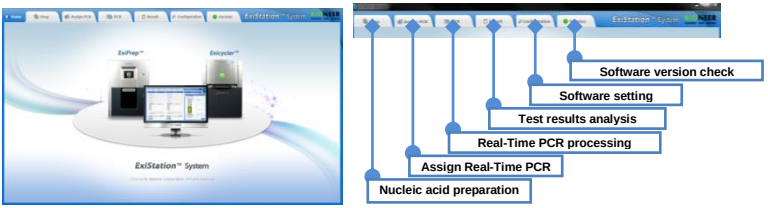


Fig. 8 Tab function of *ExiStation*™ Manager software

8) Click the '**Prep**' tab on the upper left of the main screen to initiate the nucleic acid extraction process.

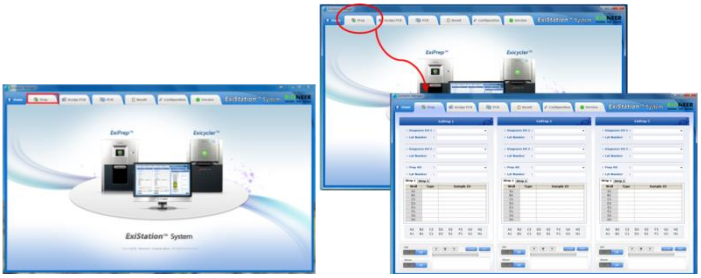


Fig. 9 Prep control panel consist of 5 panels

Prep control panel consist of 5 panels.

Instruments status panel – Status of *ExiPrep*™16 Dx
Kit selection panel –Select/enter the diagnostic kit, prep kit, and lot (or scanning the barcode of kits) information
Sample and control information panel – Enter control (NTC, PC, SPC) and sample (or scan the barcode of sample) information
Well information panel – Represent the well information with different color
***ExiPrep*™16 Dx control panel** – Control button of *ExiPrep*™ 16 Dx including UV controller, Store controller, Running controller, MISC set controller

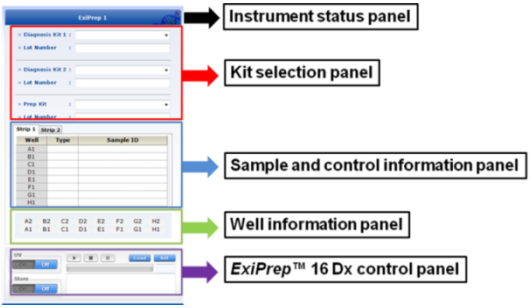


Fig. 10 Prep control panel of *ExiStation*™ Manager software

9) Click the pull-down arrow for 'Diagnosis Kit 1'.A popup will appear and select 'HCV-1211' from the pull-down menus.

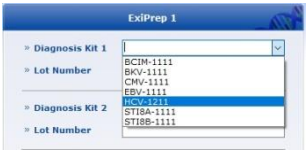


Fig. 11 Selection of Diagnostic kit

10) After selecting the 'Diagnosis Kit', a popup will appear. Inspect the Buffer Cartridge and mark the used well by clicking on the corresponding location to exclude the used well from sample assignment. Select 'OK' to finish.

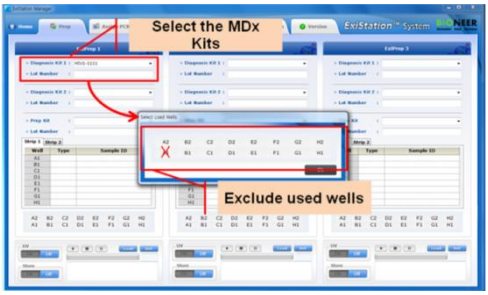


Fig. 12 'Prep' Pop-up window of *ExiStation*™ Manager software

11) Click the pull-down arrow for 'Prep Kit'. A popup of the appropriate "Prep Kit" for the selected diagnostic kit will be automatically appear. Select 'Prep Kit' from the pull-down menus.

12) Enter lot number of the diagnostic kit and the prep kit. The program will automatically allocate the NTC and SPC (or PCs) wells.
The lot of diagnostic kit and/or extraction kit is new, the program automatically assigns the NTC and SPC 1 to 5. When same lot combination of diagnostic kit and extraction kit are used to previous assay, the standard curve is automatically saved and only 1 LPC (Low Positive Control) and 1 HPC (High Positive Control) are assigned as a positive control.

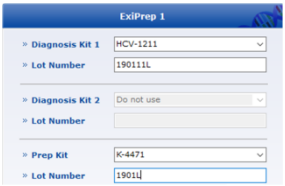


Fig. 13 Entering lot number

13) Click the 'Sample ID' column and enter sample information either using a barcode reader (optional) or type in manually.

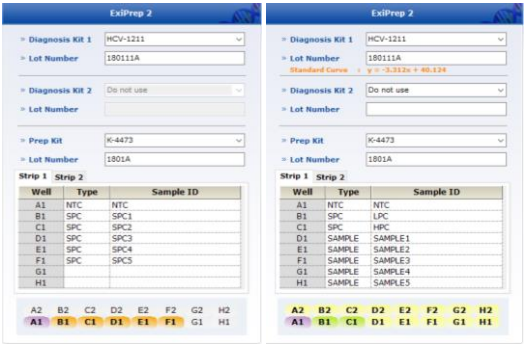


Fig. 14 Enter Sample ID (First assay/Repeated assay)

Part 2. Nucleic acid extraction by *ExiPrep*™ 16 Dx

1) Bioneer recommend the using the BSC (Class II) and clean bench for *ExiStation*™ system operation.

2) Clean the surface (preferably a positive pressure BSC) where work will be performed.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite in distilled or deionized water and rinse with distilled water or 70% EtOH, before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
>
> ⚠ **Turn off the UV lamp when using the BSC.**

3) Prepare of nucleic acid extraction kit in PCR station 1.

Table 1. List of necessary components for nucleic acid extraction

No.	contents	
1	Multi-Puncher	Optional
2	Setup Tray	*ExiPrep*™ 16 Dx
3	6-Hole Puncher	*ExiPrep*™ 16 Dx
4	Elution Tube Rack	*ExiPrep*™ 16 Dx
5	Disposable Filter Tip Rack	*ExiPrep*™ 16 Dx
6	Sample Tube Rack	*ExiPrep*™ 16 Dx
7	Buffer Cartridge ①&②	Extraction Kit
8	Disposable Filter Tip	Extraction Kit
9	Elution Tubes	Extraction Kit
10	Elution Tube Caps	Extraction Kit
11	Protection Cover	Extraction Kit
12	Waste Tray	Extraction Kit
13	Contamination shield filter paper	Extraction Kit
14	Sample Loading Tube	Extraction Kit
15	Sample Loading Tube_RNA IPC	Optional

4) Remove the shrink-wrap enclosing the both Buffer Cartridges ① and ② then remove the lids.

> ⚠ **Inspect the wells of the Buffer Cartridges and make sure all liquids are at the bottom of wells.**

Fig. 15 Remove the lids

5) Punch the film with the Hole Puncher according to the layout mapped on the software.

⚠	Since improper punching of film may cause malfunction of the instrument. Push in Hole Puncher firmly to ensure that Buffer Cartridge is punched properly.

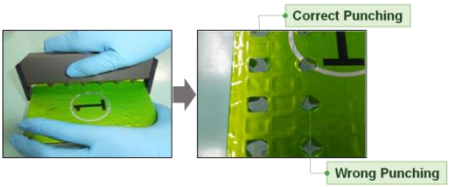


Fig. 16 Punch the film with the Hole Puncher

6) Cover Buffer Cartridges ① and ② with the lids after film punching is complete.

7) Place Buffer Cartridges on the setup tray.

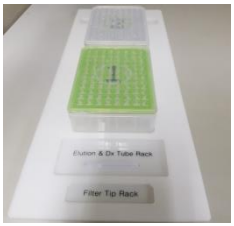


Fig. 17 Install buffer cartridge on the set-up tray

8) Take the necessary number of strips of the Diagnostic Kit Tubes from the freezer. Remove the foil covering the tubes. Insert appropriate numbers of Diagnostic Kit Tubes into the Elution Tube Rack. We recommend marking each strip of the diagnostic tubes with the corresponding column number.

⚠	You MUST make sure that the diagnostic tubes are marked so they can be identified later.
⚠	At the bottom of the Elution Tube Rack, there is a groove fitted to the *ExiPrep*™16 Dx instrument. When viewed from above, place the groove side downwards and insert the premix tubes into two upper rows.

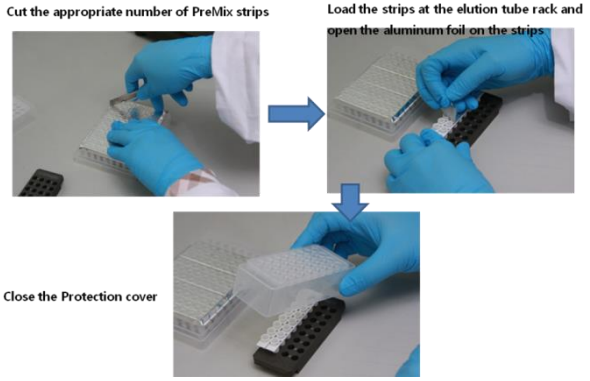


Fig. 18 Inserting the *AccuPower*® Diagnostic Kit tubes into Elution Tube Rack

9) Fasten the Protection Cover onto the Elution Tube Rack. Place Elution Tube Rack (containing Diagnostic Kit) on the setup tray.

10) Load the appropriate number of disposable filter tips at the disposable tip rack.

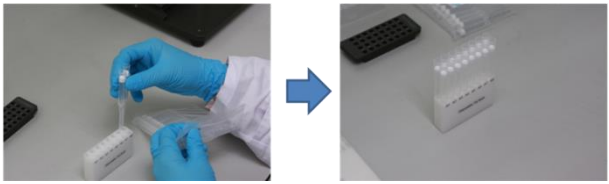


Fig. 19 Load the disposable filter tips at the disposable tip rack

11) Place the disposable tip rack on the setup tray.

12) Place the waste tray on the setup tray.

Fig. 20 Install Disposable filter tip and sate tray to the setup tray

13) Open the door of the *ExiPrep*™ 16 Dx (A-5050) and pull the Base Plate out completely. Starting from the Buffer Cartridges, place each component one-by-one into the Base Plate as described below.

14) Place the Buffer Cartridge ② on the heating block of the base plate.

> ⚠ If Buffer Cartridge ② is not properly placed on the heating block, it results in experiment failure or an instrument malfunction.

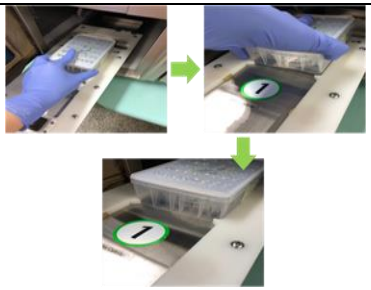


Fig. 21 Place the Buffer Cartridge ②

15) Place the Buffer Cartridge ① on the base plate.

> ⚠ Place the Buffer Cartridge ① slightly tilting the cartridge to the left side of the base plate and press the right-hand side of the cartridge firmly

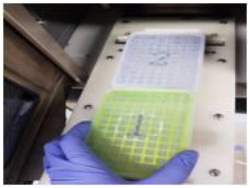


Fig. 22 Place the Buffer Cartridge ①

16) Place the Elution Tube Rack and Disposable Tip Rack on the base plate.

> ⚠ Check the Protection Cover is properly secured on the Elution Tube
> ⚠ Make sure the tips, holes and tubes are in alignment.

Fig. 23 Inserting the Disposable Tips into the Disposable Tip Rack

17) Place the Waste tray in between the Sample Tube Rack and the Buffer Cartridge ②.

⚠	Be caureful not to tip over the sample tube Rack.

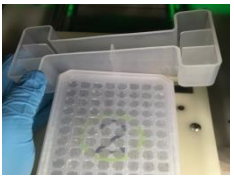


Fig. 24 Loading the Waste tray

18) Slide the Base Plate in and close the door of the *ExiPrep*™ 16 Dx. Keep the door closed until the Sample Loading Tube is ready.

⚠	When slide the base plate in, gently push the base plate not to spill the samples and reagents.

19) Prepare of clinical samples, sample loading tubes and controls in BSC. Clean the negative pressure BSC on which the nucleic acid extraction preparation will be performed.

⚠	Clean the surface with 0.5% sodium hypochlorite in distilled or deionized water and rinse with distilled water or 70% EtOH, before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.
⚠	Turn off the UV lamp when using the BSC.

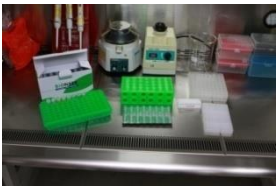


Fig. 25 Necessary components preparing for sample loading

20) Take out the RNA IPC Sample Loading Tubes from the packaging, mark it with sample name and insert them into the rack.

⚠	Before using a Sample Loading Tube, bottom of Sample Loading Tube MUST BE check for Yellow color (Dried IPC for RNA)

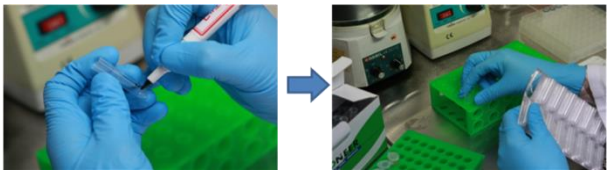


Fig. 26 Preparing Sample Loading Tube

21) Take the original clinical sample containers and controls (NTC and SPC) and pipette into the RNA IPC Sample Loading Tubes by following steps 22) ~ 25).

22) Add 400㎕ of NTC into a tube assigned as NTC. (supplied with the *AccuPower*® Diagnostic Kit)

23) Additionally add 400㎕ SPC 1~5 into the appropriate SPC wells. (supplied with the *AccuPower*® Diagnostic Kit)

> ⚠ **If you have the pre-date of same lots of Diagnostic kit and Extraction kit, you may skip SPC calibration. By the Standard information save automatically, in this case NTC, LPC and HPC role as control.**
> **When the assay is repeated with the same lot of Diagnostic kit and Extraction kit**
>
> **NTC**: Load SL buffer **400㎕ in NTC tube.**
> **LPC**: Load LPC **400㎕** (**blue cap tube**, component of *AccuPower*® Diagnostic Kit)
> **HPC**: Load HPC **400 ㎕** (**red cap tube**, component of *AccuPower*® Diagnostic Kit)

24) Move the filled Sample Tube into the Sample Tube Rack.

> ⚠ **Insert the Sample Tubes vertically to prevent spilling.**

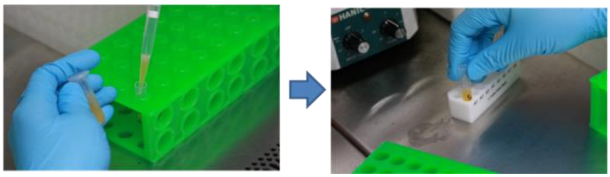


Fig. 27 Load clinical sample to Sample Loading Tube

25) Uncap clinical sample container and pipette 400 ㎕ sample into RNA IPC Sample Loading Tube. Move the RNA IPC Sample Loading Tube into Sample Tube Rack when it is filled with sample.

26) Repeat the sample loading steps individually until all samples are loaded.

⚠ If for any reason glove or tip contamination by sample is suspected, immediately exchange gloves or a tip to prevent contamination of samples.

27) Remove the waste tray on the base plate.

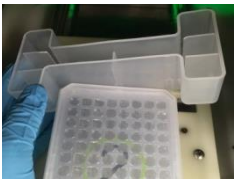


Fig. 28 Remove the waste tray

28) Load the sample tube rack on the *ExiPrep*™ 16 Dx base plate.

Fig. 29 Install of Sample Tube Rack

29) Place the Waste tray in between the Sample Tube Rack and the Buffer Cartridge ②.

⚠ Be careful not to tip over the Sample Tube Rack.

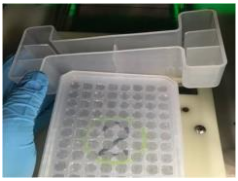


Fig. 30 Re-load Waste Tray

30) All materials are loaded.

31) Remove the lids from Buffer Cartridges.

⚠ Make sure the lids of the Buffer Cartridges are removed and all components are in the correct position.

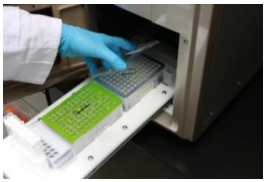


Fig. 31 Remove the lids

32) Check whether all accessories are loaded properly.

⚠	Make sure the tips, holes and tubes are all in alignment.

33) Push the base plate carefully and close the door.

⚠	When slide the base plate in, gently push the base plate not to spill the samples and reagent.

Part 3. Running *ExiPrep*™ 16 Dx and *Exicycler*™ 96 using *ExiStation*™ manager software

*** Please refer to the Equipment User Guide for basic instructions on using *Exicycler*™96 and *ExiStation*™ Manager software.**

1) Click the 'RUN (▶)' button of the ***ExiStation*™** Manager Software. Double check whether all accessories are loaded properly according to the 'Check ***ExiPrep*** Setting' list and check the boxes. Click 'OK' button to initiate the prep process.

⚠	**Nucleic acid extraction process takes 80~100 minutes according to the type of nucleic acid.**
⚠	**If any error messages appear during the extraction process. contact your local Bioneer`s distributor or headquarter for technical assistance.**

Fig. 32 Click the 'RUN' button on *ExiStation*™ Manager software

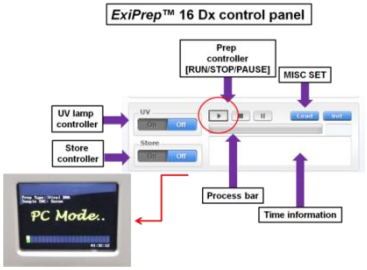


Fig. 33 *Exiprep*™ 16 Dx control panel

2) When nucleic acid extraction process is finished, the cooling block is automatically turned off. Open the door of *ExiPrep*™16 Dx (A-5050) when nucleic acid extraction process is complete, and remove Elution Tube Rack.

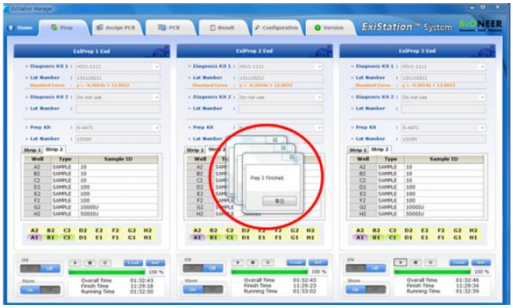


Fig. 34 Pop-up message for extraction finished

3) Move the elution tube rack to PCR station 2.

Fig. 35 PCR preparation

4) Please remove Protection Cover according to Protection Cover Separation Tool utility method.

> ⚠ **When nucleic acid extraction is finished, next step should be progressed within 10 minutes. If not, this may lead to an inaccurate result.**

① Take out Elution Tube Rack from *ExiPrep*™16 Dx and place it on top of Protection Cover Separation Tool.
Note: When placing Elution Tube Rack on Protection Cover Separation Tool, the lever must be facing left-hand side.

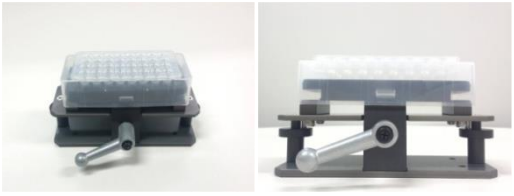


Fig. 36 Picture of Elution Tube Rack on top of Protection Cover Separation Tool

② Firmly hold down Protection Cover and Separation Tool with one hand. Rotate the lever in a clockwise 180° with the other hand.
Note: Rotate the lever until Elution Tube Rack is firmly fixed to Protection Cover Separation Tool.

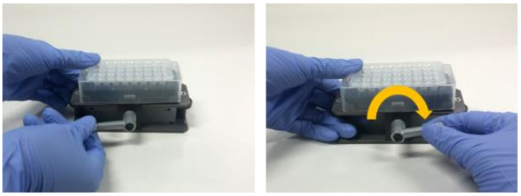


Fig. 37 Picture of lever rotation for fixing Elution Tube Rack to Protection Cover Separation Tool

③ Press down both sides of Separation Tool as shown in the picture below. This action will push Protection Cover upwards so that Elution Tube Rack can be removed with ease.
Tip: Hold down Protection Cover with one hand. Then press down each side of Separation Tool consecutively to prevent any liquid from splashing.

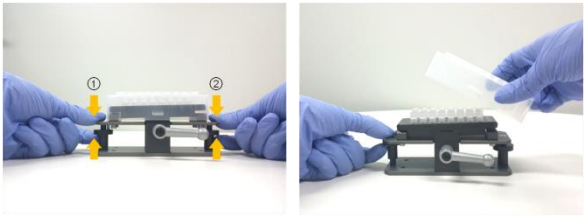


Fig. 38 Picture of pressing down each side of Separation Tool and removing Protection Cover from Separation Tool

5) Seal PCR Tube using Optical sealing film and then proceed to next step. For more information on Sealing process, refer to step 6).

6) Seal the Diagnostic Tubes with the adhesive Optical Sealing Film.

⚠ In order to avoid contaminations and invalid results, seal all the tubes thoroughly.
⚠ Store the sealed diagnostic tubes at 4°C until use (if the prep reaction is divided into 2 steps, store it until 2nd prep finishes).

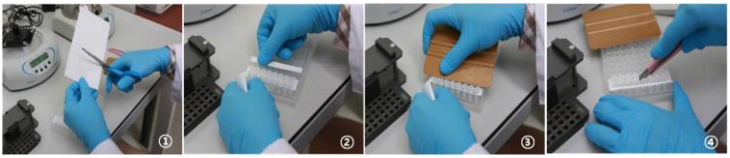


Fig. 39 Seal PCR premix strip

7) Right before the PCR reaction, completely mix the tube contents using ***ExiSpin™*** (A-7040). (*ExiSpin*™ parameters: 2500rpm for 1 sec., Hard vortex for 20 sec./ 20 cycles)

⚠ Bioneer's PCR premix contains vacuum-dried PCR reagents. Insufficient mixing could result in invalid PCR results, so mix until the premix is thoroughly dissolved.
⚠ MAKE SURE TO mark each diagnostic kit to prevent mix up.
⚠ When nucleic acid extraction is finished, next step should be progressed within 10 minutes. If not, this may lead to inaccurate result.

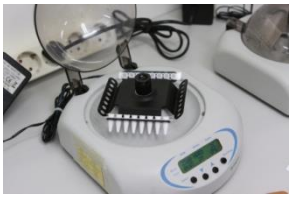


Fig. 40 Mix the PCR Premix Strip using *ExiSpin*™

⚠ DO NOT manipulate *ExiSpin*™ protocol, arbitrarily
⚠ HAVE TO adjust balance

8) While *ExiSpin*™ is operating, turn on *Exicycler*™ 96. Turn on the Standby Power Switch, located at the rear of the instrument. LED status light on the front of instrument, should turn on Blue. Press the Power Switch for 3 seconds. A brief self-test sequence will initiate. When self-test complete, LED will blink GREEN with a short beep.

Fig. 41 Operation button (door button, power button and status LED) of *Exicycler*™ 96

9) Click the 'Assign PCR' tab on the main screen of *ExiStation*™ Manager program. 'Assign PCR tab' consist of six tabs.

Assign	Assign the Prep WorkList on 96 well plate, marked the strip number.
Current Step	It indicates the progress of nucleic acid extraction in *ExiPrep*™16 Dx. Prep: middle of nucleic acid extracting / Prep End: Finish the nucleic acid extraction
Diagnosis Kit	In Prep WorkList, displayed the diagnostic kit that has been extracted. Selected diagnostic kit can operate PCR with other diagnostic kits at the same time.
Prep Kit	It indicates used extraction kit in prep WorkList.
Start Time	It indicates start time of nucleic acid extraction.
Finish Time	It indicates finish time of nucleic acid extraction.

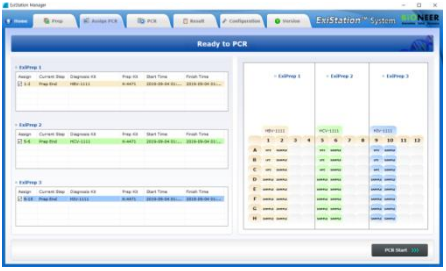


Fig. 42 PCR Random Access

10) Click 'Assign PCR' tab and check the box of each 'Prep Work List' to assign PCR position. PCR position correspond to *ExiPrep*™ 16 Dx #1~3 in order.

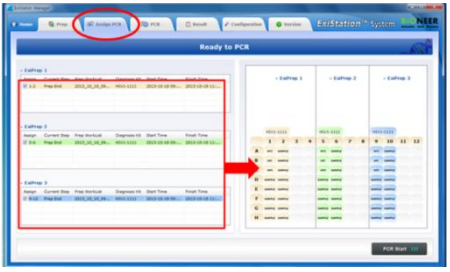


Fig. 43 'Assign PCR' tab – PCR Start

11) Push the Door Switch for 2 seconds to slide the 96-well thermal block out. Insert the reaction tubes in their locations. When sample loading is complete, push the Door Switch for 2 seconds to close the door.

⚠ **Make sure the sample loading configuration is in agreement with the assigned well position.**

⚠ If you are running less than 6 strips for a PCR run, please insert a dummy strip at the opposite end (column 12) to balance out the pressing force of the hot lid in *Exicycler*™ 96.

12) Place the mixed premix tubes into the assigned well position of *Exicycler*™ 96 when cycling is complete. For detailed operation instructions of *Exicycler*™ 96 and *ExiStation*™ Manager software, see the relevant ***User's Guide.***

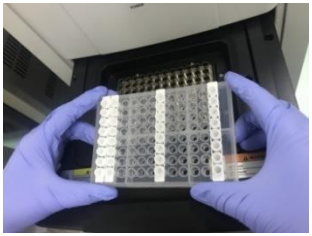


Fig. 44 Way to PCR Premix Strip setup of *Exicycler*™ 96

13) Select 'Assign PCR' tab and confirm assigned 'Prep Work List'. After the 'Prep' process, 'Current Step' will be presented as 'Prep End' and the upper status bar will be changed to 'Ready to PCR'. Initiate PCR run by clicking the activated 'PCR Start' button at the bottom right hand side of the window.
A popup window will appear prompting the user to enter a Work List Name. Click 'OK' after entering a name to generate a Work List for Real-Time PCR.

⚠ Default Work List file path is 'C: > *ExiStation*_Data > user > GUEST > WorkList'.

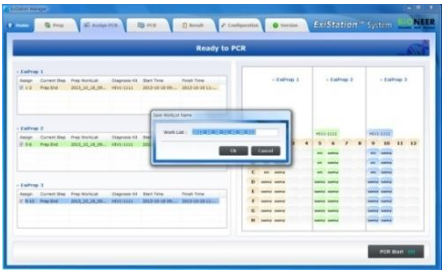


Fig. 45 Pop-up window of "Data name"

14) After entering the Work List Name, 'PCR' tab will be activated and the ***Exicycler***™ 96 will automatically initiate PCR run.

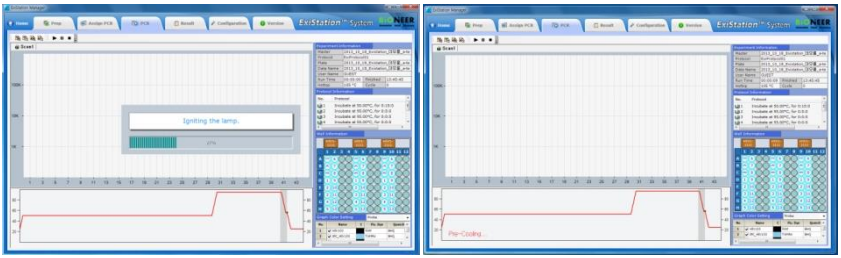


Fig. 46 PCR Running screen

15) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument and discard all liquids and consumables in their appropriate containers.

⚠	If un-used wells are present in the Buffer Cartridges, take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a positive pressure BSC for later use.
⚠	Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

16) Press the 'Misc Set' button, remove Tip Protector and Contamination Shield then press the 'Misc Set' button again

17) Push the Base Plate in, shut the instrument door and initiate UV sterilization by clicking "UV ON" on the control panel.

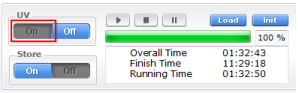


Fig. 47 *ExiPrep*™16 Dx control panel – UV

18) After the PCR run is finished, select 'Result' tab to check the results of each samples.

⚠	Click 'Analysis' button to open the dedicated analysis popup which presents detailed results including a fluorescence graph.
⚠	DO NOT peel off an optical sealing firm from Diagnostic Kit. Discard them according to local safety regulations or internal laboratory procedure.

Fig. 48 Result analysis using *ExiStation*™ Manager software

19) The result data files are saved in 'C: > *ExiStation*_Data > user > GUEST > WorkList > relevant data file name' folder.

8.5 Experimental procedure II (*ExiStation*™ 48, *ExiStation*™ 48A)

Part 1. Assigning test using *ExiPrep*™48 software

*** Please refer to user's guide of *ExiPrep*™ 48 Viral DNA/RNA Kit, *ExiPrep*™ 48 Dx or *ExiLT* for basic workflow.**

1) Turn on the *ExiPrep*™ 48 Dx. Switch on back of instrument, press the POWER button on the front of instrument over 1 second.

2) As it starts initialization by itself, the LCD screen will automatically appear.

3) When the initialization of instrument is completed, the main screen appears on the LCD screen. If initialization is NOT successfully completed, contact us (Bioneer) or agencies.

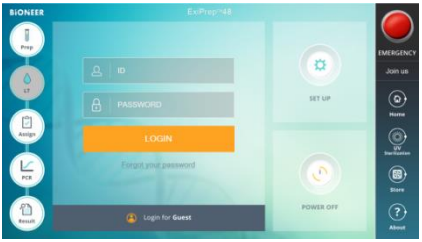


Fig. 49 Main screen of *ExiPrep*™ 48 Dx

4) Main screen consists of 5 icons.

Prep – Set-up and control nucleic acid extraction(*ExiLT*™, *ExiPrep*™48 Dx)
LT – Automatic de-capping and liquid transfer system
Assign – Extracted information can be displayed
PCR – Monitoring extraction of Real-Time PCR (*Exicycler*™ 96)
Result – Show the results after executing PCR

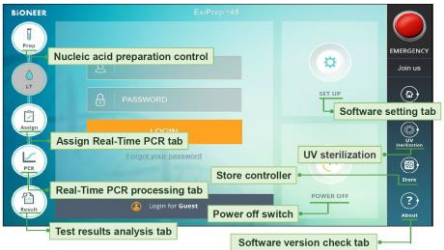


Fig. 50 Icons function of *ExiPrep*™48 software

5) Log in with the registered ID. When you log in as a guest, it is usually saved data in the folder specified. If you log in with your ID, you can specify a folder to store so that you can manage the resulting data more efficiently (optional).

6) Before the Prep, separate the Contamination Shield from *ExiPrep*™ 48 Dx. And clean with 70% EtOH and attach the Contamination Shield filter paper then install the prepared Contamination Shield.

Fig. 51 Decontamination step-1 ; Separation and installation of the Contamination Shield

7) Close the door of the instrument, Click the UV sterilization icon. And select the '15 Minutes' button (Turn on the UV sterilization). After 15 minutes, UV sterilization turns off automatically.

Fig. 52 Decontamination step-2 ; UV sterilization

8) Touch the Prep icon on the main screen. Screen will be changed as shown below figure. Enter Prep mode for nucleic acid extraction by touching the ExiStation.

Fig. 53 Initial screen of Prep tab

9) Touch the pull-down arrow of Diagnosis Kit 1, show a list of available diagnosis kits. Press the HCV-1211.

Fig. 54 Entering Diagnosis Kit information

10) As pop-up “Select Lane & Well” message, select the well to use. Later check the already used well of Buffer cartridge ①. Click the used well, appear “X” sign upper that well. Finally click the “OK” button. If there are not used well, click the “OK” button straight.

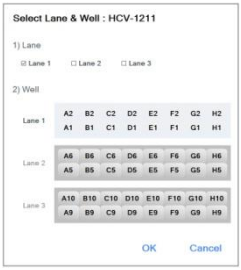


Fig. 55 Screen of ‘Select Lane & Well’

11) Enter Lot number of the diagnosis kit.

12) Touch the pull-down arrow of Prep Kit. Show the prep kit to use. Select the prep kit to use then enter Lot information of Prep Kit.

Fig. 56 Entering Kit information

13) As pop-up “Sample Type” message, select the sample to use.

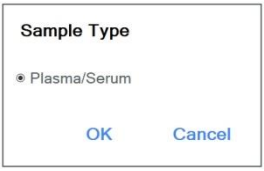


Fig. 57 Screen of 'Sample Type'

14) If either Lot number for Diagnosis Kit or/and Prep Kit is new, Notification window required Standard Calibration will be displayed.

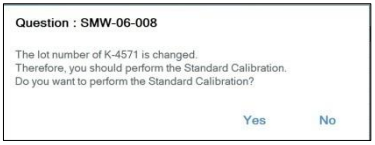


Fig. 58 Pop-up message for Standard Calibration process

15) NTC and SPC position are automatically displayed on remaining well, primary setting is NTC and each one of SPC 1~5. Repeat the same Lot number of Extract/Diagnosis kit's standard curve saves already. In this case LPC and HPC is set one well for each instead of SPC 1~5.

16) Complete generate of Standard curve normally, proceed the next experiment using clinical samples. If the experiment works, appear several information; standard curve, right position of NTC/LPC/HPC. Then click the 'Sample ID', input the clinical sample's information. (Optional-using barcode reader)

Fig. 59 Entering sample information

Part 2. Nucleic acid extraction by *ExiPrep*™ 48 Dx

1) It is recommended that handling clinical samples, all related works should be conducted within a negative pressure BSC (Class II) for user's safety and prevention contamination.

2) Clean the BSC and check that all necessary components for extraction and sample before nucleic acid extraction. Prepare extraction components within a positive pressure BSC. Recommend to perform at separated place referring to 8.1.

> ⚠ Clean the surface with 0.5% sodium hypochlorite and 70% ethanol or D.I water before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.
> ⚠ It must be turn off the UV lamp while using the BSC.

3) Check that all necessary components are present before proceeding and perform operation within positive pressure BSC-1.

Table 3. List of necessary components for nucleic acid extraction

Prep tools	Consumables
■ Setup Tray ■ Hole Punch ■ Sample Tube Rack ■ Elution Tube Rack ■ Clamp	■ Buffer Cartridges ① and ② ■ Sample Loading Tubes_IPC ■ Disposable Tips & Rack ■ Elution Tubes ■ Elution Tube Caps ■ Waste Tray ■ Contamination shield filter paper

4) Remove the shrink-wrap enclosing the both Buffer Cartridges ① and ② within positive pressure BSC-1.

> ⚠ Inspect the wells of the Buffer Cartridge and make sure all liquids are at the bottom of the wells.

5) Take the necessary number of *AccuPower*® Diagnostic Kit tube from the freezer and insert diagnostic kit tube into the elution tube Rack. Remove the covered foil of diagnostic kit tube. Mark each strip of the diagnostic tubes with the corresponding column number.

> ⚠ You MUST make sure that the diagnostic tubes are marked so they can be identified during the process.
> ⚠ At the bottom of the elution tube rack, there is a groove fitted to the *ExiPrep*™ 48 Dx instrument. When viewed from above, place the groove side downwards and insert the premix tubes into two upper rows as shown below figure.

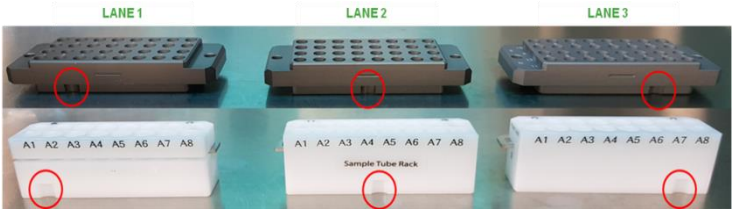


Fig. 60 Checking the position of Elution Tube Rack and Loading Tube Rack

6) Fasten the protection cover onto the elution tube rack.

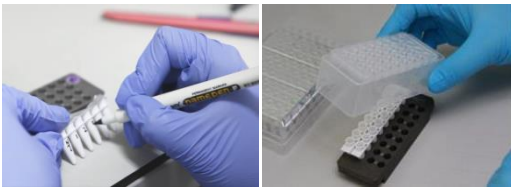
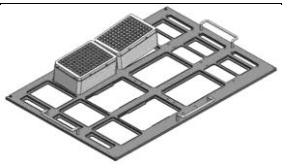
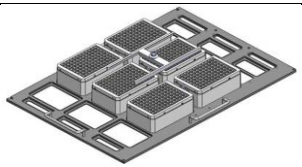
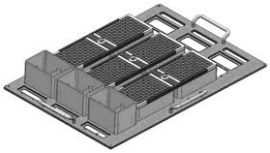
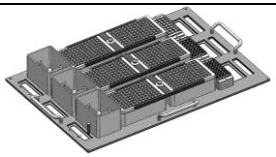
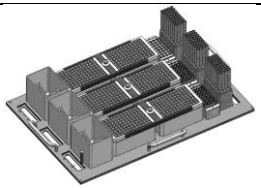
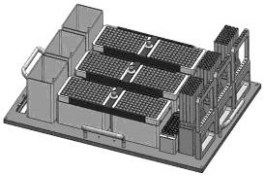


Fig. 61 Installing protection Cover

7) Open the door of the instrument (*ExiPrep*™ 48 Dx (A-5150)), remove set-up tray installed inside and place it on a flat experiment bench.

①		Install buffer cartridge ①, ② to the sample quantity on the set-up tray.
②		Install clamp on top of the buffer cartridge. Clamps must be installed per lane. And hold the clamp.
③		Install waste tray.

④		Install elution tube rack that installed PCR Premix Strip and protection cover to the set-up tray.
⑤		Remove the sticker in center of Disposable Tip Rack and install it to the Setup Tray.
⑥		Install 8-hole punch.

8) Completed installing components for nucleic acid extraction, prepare control and samples.

9) Prepare of clinical samples in negative pressure BSC. Before using clean the BSC on which the nucleic acid extraction will be performed. Perform sample within a negative pressure BSC, clean the BSC before using.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite and 70% ethanol or DI water before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
> ⚠ **It must be turn off the UV lamp while using the clean bench.**

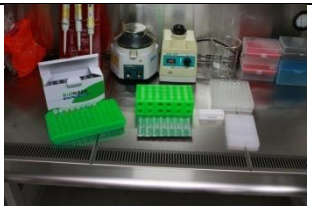


Fig. 62 Preparing for sample loading

10) Take necessary number of Sample Loading Tube, mark the name on the sample loading tube to prevent confusion. Insert them into the rack.

> ⚠ **Before using a Sample Loading Tube, bottom of Sample Loading Tube MUST BE check for Yellow color (Dried IPC for RNA)**

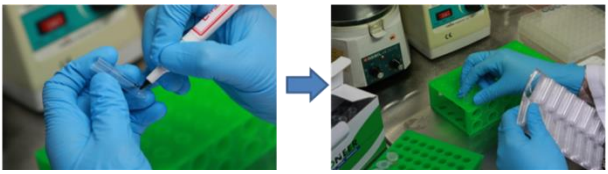


Fig. 63 Preparing Sample Loading Tube

11) Prepare of container for sample and control (SL buffer, SPC, LPC/HPC), perform to loading into sample loading tube follow next 12) ~ 15) step.

12) Add 400 ㎕ of NTC into a tube that is assigned as NTC. (supplied with *AccuPower*® Diagnostic Kit)

13) Additionally add 400 ㎕ SPC1~5 into the appropriate SPC wells. (supplied with the *AccuPower*® Diagnostic Kit)

> ⚠ If you have the pre-date of same lots of Diagnostic kit and Extraction kit, you may skip SPC calibration. By the Standard information save automatically, in this case NTC, LPC and HPC role as control.
> When the assay is repeated with the same lot of Diagnostic kit and Extraction kit
>
> NTC: Load SL buffer **400㎕ in NTC tube.**
> LPC: Load LPC **400㎕** (**blue cap tube**, component of *AccuPower*® Diagnostic Kit)
> HPC: Load HPC **400 ㎕** (**red cap tube**, component of *AccuPower*® Diagnostic Kit)

14) Ready to use the Sample loading tube loaded product's control, install the Sample Tube Rack

> ⚠ After unlocking the sample tube rack's fixing device, set the tube.
> ⚠ When the Sample Tube Rack install, keep vertical direction during removal and installation of the rack to prevent pour of loaded solution

15) Load 400 ㎕ of clinical sample to Sample Loading Tube. Finish the clinical sample loading, move the Sample Loading Tube to Sample Tube Rack.

> ⚠ Confirm the exact position of each Sample Loading Tube, and then set up.
> ⚠ If gloves or tip and so on are contaminated by clinical sample, remove the pollutant immediately. Then use new one.
> ⚠ Once the tube has been installed, push the fixing device to lock Sample Loading Tube's position

16) Place the Sample Tube Rack on *ExiPrep*™ 48 Dx setup tray.

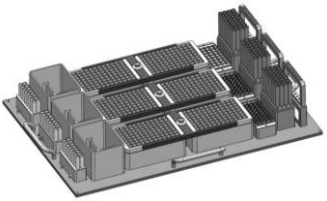


Fig. 64 Install of Sample Tube Rack

17) Check all components are installed normally on Setup tray.

18) Install the Setup tray on the *ExiPrep*™ 48 Dx instrument.

> ⚠ Check the each side, Left: Sample Tube Rack / Right: 8 hole punch, Then push the Setup tray into the instrument, carefully.

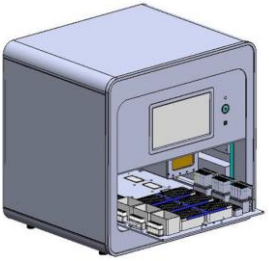


Fig. 65 Install of Setup tray

19) Finish the all process, – setting the program, ready to sample and install the setup tray– Click the "Apply Run" screen located right bottom to start extract the nucleic acid.

> ⚠ Running time of extraction takes 60~80 minutes according to sample type.
> ⚠ During extraction process, If an error message occurs please contact the nearest store or Bioneer International Molecular Diagnosis TS team.

Fig. 66 Start extraction of nucleic acid though *ExiPrep*™ 48 software

Part 3. Running *ExiPrep*™ 48 Dx and *Exicycler*™ 96 using *ExiPrep*™48 software

*** Please refer to the Equipment User Guide for basic instructions on using *Exicycler*™96 and *ExiPrep*™ 48 software.**

1) Finish the extraction of nucleic acid, you will see the pop-up message to notify the end. Press the "Door" button to open the door on the front of the machine, and take out the Setup Tray.

> ⚠ **Finish the extraction of nucleic acid, take out the Setup Tray within 10 minutes. Then separate the PCR Premix Strip from Elution Tube Rack, process after steps. The long delay can lead to degradation of nucleic acid, which may affect the result value.**

2) Refer the '8.4 Experimental procedure Ⅰ-Part 3. 4)~7), Ready to PCR process after separate the PCR Premix Strip of Elution Tube Rack.

3) Click the 'Assign' icon on the main screen of *ExiPrep*™ 48 software.
'Assign icon' consist of six tabs.

Assign	Assign the Prep WorkList on 96 well plate, marked the strip number.
Current Step	It indicates the progress of nucleic acid extraction in *ExiPrep*™16 Dx. Prep: middle of nucleic acid extracting / Prep End: Finish the nucleic acid extraction
Diagnosis Kit	In Prep WorkList, displayed the diagnostic kit that has been extracted. Selected diagnostic kit can operate PCR with other diagnostic kits at the same time.
Prep Kit	It indicates used extraction kit in prep WorkList.
Start Time	It indicates start time of nucleic acid extraction.
Finish Time	It indicates finish time of nucleic acid extraction.

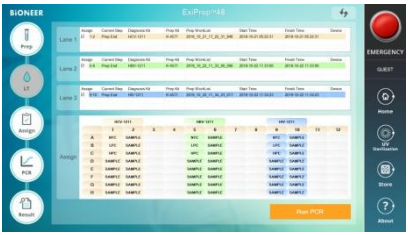


Fig. 67 PCR Random Access

4) Click the "Assign" icon. The information list of finished nucleic acid extraction appears on the screen. Check the box what you want to PCR process. According to lane position of *ExiPrep*™ 48 Dx, decide the PCR well position.

Fig. 68 Select the sample for PCR process

5) Press the Door button of *Exicycler*™ 96 for 2 seconds, 96-well thermal block gets out of instrument. Set PCR Premix Strip the right position selected by the software.

> ⚠ PCR Premix Strip position exactly match with assigned position in software.
> ⚠ If you run PCR under 4 strips, put the balance strip in opposite position to balance of *Exicycler*™ 96 thermal block.

6) After PCR Premix Strip setting, press the "Run PCR" button located in lower right. A pop-up window "Data name" appears, fill in the name of test then press "OK" button.

> ⚠ WorkList saves this way ; *ExiPrep*™ 48 software> SET UP > Data > WorkList

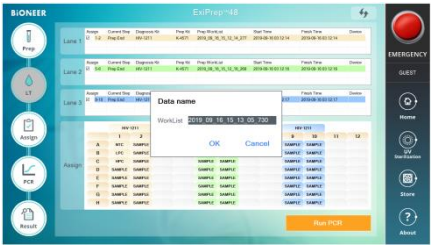


Fig. 69 Pop-up window of "Data name"

7) Complete 6) step, *Exicycler*™ 96 runs automatically.

8) Complete the PCR, Click 'Result' icon to confirm the result.

> ⚠ Click "Analysis", an analysis program appears in a pop-up window and can confirm detail result.
> ⚠ After click the "Print" button(right of Analysis button), select the analysis result what you want to print can print as report.
> ⚠ Analysis result saves automatically on this folder
> ⚠ *ExiPrep*™ 48 software> SET UP > Data > WorkList > relevant data.

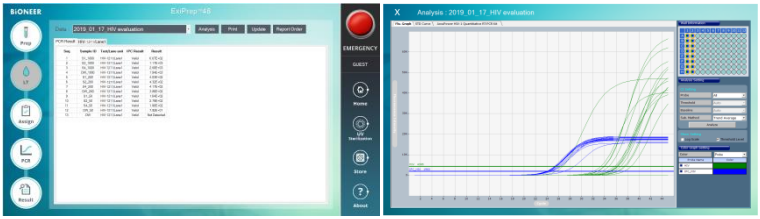


Fig. 70 Data analysis

8.6 Handling process of experimental waste

8.6.1 *ExiPrep*™ 16 Dx

1) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument and discard all liquids and consumables in their appropriate containers.

> ⚠ If un-used wells are present in the Buffer Cartridges, take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a positive pressure BSC for later use.
> ⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

2) Press the 'Misc Set' button, remove Tip Protector and Contamination Shield then Cleaning with 70% ethanol and press the 'Misc Set' button again

3) Push the Base Plate in, shut the instrument door and initiate UV sterilization by clicking "UV ON" on the control panel.

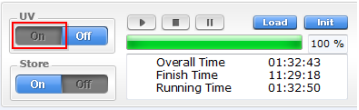


Fig. 71 *ExiPrep*™16 Dx control panel – UV

8.6.2 *ExiPrep*™ 48 Dx

1) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument and discard all liquids and consumables in their appropriate containers.

> ⚠ If un-used wells are present in the Buffer Cartridges, remove the used Filter tip from Buffer Cartridge ②. Take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a positive pressure BSC for later use.
> ⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

2) Remove Tip Protector and Contamination Shield then Cleaning with 70% ethanol and reinstall.

3) Push the Set-up tray in, shut the instrument door and initiate UV sterilization by clicking "UV ON" on the control panel.

Fig. 72 *ExiPrep*™48Dx control panel – UV

8.6.3 *Exicycler*™ 96

1) After the PCR run is finished, select 'Result' tab to check the results of each samples.

⚠	DO NOT peel off an optical sealing firm from Diagnostic Kit. discard them according to local safety regulations or internal laboratory procedure

8.7 Data Analysis

(1) Calibration (HCV SPC (1) – (5))

For test with new Lot of diagnostic kit and/or extraction kit, calibration must be performed. The test use 5 wells of SPC (HCV SPC (1) ~ (5)) to generate a standard curve. Additionally, the user can check for batch validity with *ExiStation*™ manager software either by monitor or in a printed report. The batch is valid, if at least 3 SPCs are valid.

(2) Control (HCV LPC and HPC)

Every test is accompanied with control. The test uses 2 wells of PCs (HPC, LPC) to confirm a validity of each test. The user can check the validity of test with *ExiStation*™ manager software either by monitor or in a printed report.

(3) NTC

Every test uses 1 well of NTC to check any contamination in the process of sample loading, nucleic acid extraction, PCR preparation, in order to prevent false-positive error.

The validity of SPC and NTC are determined by Ct value of HCV signal. If the assay is valid, HCV Ct will be 'undetermined' in NTC well and SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User's Guide section 10. Troubleshooting.

Table 1.Specimen results are interpreted as follows:

Titer Result (IU/㎖*)	Interpretation
Not detected	No Ct value (>45Ct) of HCV obtained. Results are reported as "Not detected".
< 2.00E+01 IU/㎖	Calculated IU/㎖are below the Limit of Quantification of the assay. Report results as "<2.00E+01".
≥ 2.00E+01 IU/㎖ and ≤ 1.00E+08 IU/㎖	Calculated results greater than or equal to 2.00E+01 IU/㎖ and less than or equal to 1.00E+08 IU/㎖ are within the Linear Range of the assay.
> 1.00E+08 IU/㎖	Calculated IU/㎖ are above the range of the assay. Results are reported as "greater than 1.00E+08 IU/㎖". If quantitative results are desired, the original specimen should be diluted with HCV-negative human EDTA-plasma or Human serum and the test repeated. Multiply the reported result by the dilution factor.

* IU/㎖; HCV RNA concentration in copy/㎖ X 1.50 IU/copy = HCV RNA in IU/㎖

8.8 Quality Control

(1) IPC (Internal Positive Control)

Every test tube contains an IPC to check PCR inhibition by the impurity or the miscontrolled thermal cycling in order to monitor the whole process. IPC is dried within Sample Loading tube (accessory for nucleic acid extraction, not provide). High concentrations of HCV RNA can lead to a reduced or absent fluorescence signal of the IPC due to PCR competition. The validity of IPC is determined by Ct value of IPC signal. If the Ct value is within specified range, it is valid. If the Ct value is out of specified range, it is invalid. The validity of SPC and NTC are determined by Ct value of HCV signal. If the assay is valid, HCV Ct will be 'undetermined' in NTC well and SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User's Guide section 10. Troubleshooting.

The result of IPC determines the validity of the test and Ct value of HCV signal determines the HCV concentration (IU/㎖) of the sample. For the high titer specimen above the desired quantitative range, the original specimen should be diluted with the SL buffer provided, and the test must be repeated.

9. PERFORMANCE CHARACTERISTICS

9.1 Analytical Characteristics

9.1.1 Limit of Detection (LoD)

The limit of detection of *AccuPower*® HCV Quantitative RT-PCR Kit was determined by analysis of serial dilutions of WHO International Standard for HCV RNA for Nucleic Acid Amplification Technology Assays (5th WHO International Standard), in HCV-negative human EDTA plasma and in HCV-negative serum. 6 serial dilutions of the panel and negative were tested with 3 lots of *AccuPower*® HCV Quantitative RT-PCR Kit.

AccuPower® HCV Quantitative RT-PCR Kit detected HCV RNA with a detection rate of 95%, as determined by PROBIT, at a concentration of 10.7 IU/㎖ in EDTA-plasma and 14.1 IU/㎖ in serum

Table 4. Detection rate of *AccuPower*® HCV Quantitative RT-PCR Kit at each concentration in EDTA-plasma

Nominal concentration		Number of replicates tested (N)	Number of positives detected (N)	Positive rate (%)
IU/ml	Log_{10}IU/㎖			
NTC	0	72	0	0
1.55	0.19	72	29	40
3.09	0.49	72	52	72
6.31	0.80	72	62	86
12.59	1.10	72	67	93
25.12	1.40	72	72	100
50.12	1.70	72	72	100

Table 5. Detection rate of *AccuPower*® HCV Quantitative RT-PCR Kit at each concentration in serum

Nominal concentration		Number of replicates tested (N)	Number of positives detected (N)	Positive rate (%)
IU/ml	Log_{10}IU/㎖			
NTC	0	72	0	0
1.55	0.19	72	30	41
3.09	0.49	72	43	59
6.31	0.80	72	54	75
12.59	1.10	72	68	94
25.12	1.40	72	72	100
50.12	1.70	72	72	100

Table 6. Limit of Detection PROBIT analysis

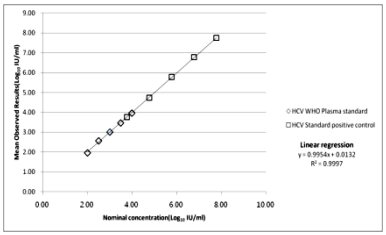
Matrix	LoD by PROBIT at 95% detection rate	(95% Confidence interval)
EDTA-plasma	10.7 IU/㎖	(7.94 ~ 14.45) IU/㎖
	1.03 Log_{10} IU/㎖	(0.90 ~ 1.16) Log_{10} IU/㎖
Serum	14.1 IU/㎖	(10.47 ~ 19.50) IU/㎖
	1.15 Log_{10} IU/㎖	(1.02 ~ 1.29) Log_{10} IU/㎖

9.1.2 Traceability

The Traceability study of the *AccuPower*® HCV Quantitative RT-PCR was determined by testing the WHO 5th HCV International Standard panel (NIBSC code: 14/150, UK) containing 5.00 Log_{10} IU/㎖ of HCV, genotype 1a and HCV Standard Positive Control two dilutions of international standard panel, 7dilutions of Standard Positive Control and one dilution(9.00 Log_{10} IU/㎖) of virus particle was tested.

All material demonstrated co-linear dilution performance across the linear range of *AccuPower*® HCV Quantitative RT-PCR Kit. According to these results, quantification value for HCV international standard positive panel, Standard Positive Control and virus particle was similar to the expected value with the results of Deviation from linearity value within 0.2 Log_{10} IU/㎖

EDTA-plasma



Serum

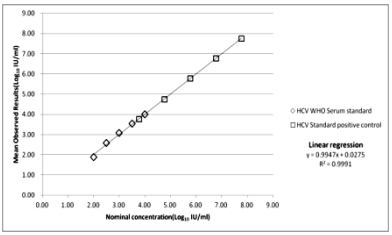


Fig. 73 Traceability to WHO international standard panel

9.1.3 Verification of limit of detection for HCV genotype 1-6

The verification of limit of detection in *AccuPower*® HCV Quantitative RT-PCR Kit for HCV genotype 1-6 was determined by analysis of 6 serial dilutions of HCV RNA Genotype AccuTrak™ Qualification Panel.

20-replicate was performed in each dilutions and the study results demonstrate that *AccuPower*® HCV Quantitative RT-PCR Kit can detect HCV RNA in EDTA plasma at a concentration as low as 1.4 Log_{10} IU/㎖, with a positivity rate greater than or equal to 95%.

Table below demonstrate result of probit analysis for genotypes 1b, 2a, 2b, 3, 4, 5, 6. *AccuPower*® HCV Quantitative RT-PCR Kit can detect HCV genotype LoD in EDTA plasma and Normal Human serum.

Table 7. Detection rate of *AccuPower*® HCV Quantitative RT-PCR Kit at each concentration in EDTA-plasma

Genotype	Concentration (Log_{10} IU/㎖)	Number of replicates tested (N)	Number of positive detected (N)	Positive rate (%)
1b	1.70	20	20	100
	1.40	20	20	100
	1.10	20	20	100
	0.80	20	20	100
	0.49	20	14	70
	0.19	20	9	45
2a	1.70	20	20	100
	1.40	20	20	100
	1.10	20	18	90
	0.80	20	16	80
	0.49	20	15	75
	0.19	20	5	25
2b	1.70	20	20	100
	1.40	20	20	100
	1.10	20	17	85
	0.80	20	17	85
	0.49	20	5	25
	0.19	20	8	40
3	1.70	20	20	100
	1.40	20	20	100
	1.10	20	18	90
	0.80	20	16	80
	0.49	20	12	60
	0.19	20	5	25
4	1.70	20	20	100
	1.40	20	20	100
	1.10	20	19	95
	0.80	20	17	85
	0.49	20	13	65
	0.19	20	7	35
5	1.70	20	20	100
	1.40	20	20	100
	1.10	20	19	95
	0.80	20	12	60
	0.49	20	7	35
	0.19	20	7	35
6	1.70	20	20	100
	1.40	20	20	100
	1.10	20	19	95
	0.80	20	13	65
	0.49	20	10	50
	0.19	20	6	30

Table 8. Detection rate of *AccuPower*® HCV Quantitative RT-PCR Kit at each concentration in Serum

Genotype	Concentration (Log_{10} IU/㎖)	Number of replicates tested (N)	Number of positive detected (N)	Positive rate (%)
1b	1.70	20	20	100
	1.40	20	20	100
	1.10	20	20	100
	0.80	20	20	100
	0.49	20	14	70
	0.19	20	9	45
2a	1.70	20	20	100
	1.40	20	20	100
	1.10	20	20	100
	0.80	20	16	80
	0.49	20	10	50
	0.19	20	11	55
2b	1.70	20	20	100
	1.40	20	20	100
	1.10	20	16	80
	0.80	20	11	55
	0.49	20	7	35
	0.19	20	3	15
3	1.70	20	20	100
	1.40	20	20	100
	1.10	20	20	100
	0.80	20	15	75
	0.49	20	11	55
	0.19	20	8	40
4	1.70	20	20	100
	1.40	20	20	100
	1.10	20	19	95
	0.80	20	17	85
	0.49	20	11	55
	0.19	20	7	35
5	1.70	20	20	100
	1.40	20	19	95
	1.10	20	18	90
	0.80	20	12	60
	0.49	20	9	45
	0.19	20	7	35
6	1.70	20	20	100
	1.40	20	19	95
	1.10	20	16	80
	0.80	20	14	70
	0.49	20	13	65
	0.19	20	5	25

Table 9. Genotype LoD summary results of *AccuPower®* HCV Quantitative RT-PCR Kit by manufacture

Matrix	Genotype	LoD (Log_{10} IU/㎖)	95% CI (Log_{10} IU/㎖)
EDTA-plasma	1b	0.73	0.54 – 0.93
	2a	1.05	0.75 – 1.35
	2b	1.26	1.01 – 1.51
	3	1.14	0.90 – 1.38
	4	1.14	0.85 – 1.43
	5	1.26	1.02 – 1.50
	6	1.20	0.96 – 1.44
Serum	1b	0.91	0.68 – 1.14
	2a	1.08	0.82 – 1.34
	2b	1.35	1.13 – 1.57
	3	1.09	0.85 – 1.33
	4	1.09	0.83 – 1.35
	5	1.33	1.07 – 1.59
	6	1.40	1.11 – 1.69

9.1.4 Linear range and Limit of Quantification (LoQ)

Linearity and LoQ of main genotype 1a was performed with serial dilution of WHO 5th HCV International Standard panel (NIBSC code: 14/150, UK). For low titer members and high tier member with *AccuPower®* HCV Quantitative RT-PCR Kit.

8 dilutions of each panel (4 dilutions) and HCV virus particle (4 dilutions) from 8.00 log_{10} IU/㎖ to 1.18 log_{10} IU/㎖in EDTA-plasma and from 8.00 log_{10} IU/㎖ to 1.30 log_{10} IU/㎖in Human Serum for HCV genotype 1a were prepared. Three (3) dilution of each panel from 3.00 log_{10} IU/㎖ to 1.18 log_{10} IU/㎖ in EDTA-plasma and from 3.00 log_{10} IU/㎖ to 1.18 log_{10} IU/㎖ in serum for other HCV genotypes were prepared.

The evaluation of main LoQ and linearity was performed with three (3) different lots of *AccuPower®* HCV Quantitative RT-PCR Kit. Test was perform with each concentration in two (2) replicates and two (2) runs per day. Test was performed over the period of four (4) different days and in three (3) different *ExiStation*™ system. Resulting in forty (40) data per dilution.

Linear range for *AccuPower®* HCV Quantitative RT-PCR Kit was from 1.18 Log_{10} IU/㎖ to at least 8.00 Log_{10} IU/㎖ in EDTA-plasma and1.30 Log_{10}IU/㎖ to at least 8.00 Log_{10} IU/㎖ in Human serum. Maximum deviation between the observed mean Log_{10}titer and the best fitted 1st-order model less than 0.20 Log_{10} IU/㎖for each concentration level tested in this interval. Therefore, result of this study support linear range of 1.18 Log_{10} IU/㎖ to at least 8.00 Log_{10} IU/㎖ in EDTA-plasma and 1.30 Log_{10}IU/㎖ to at least 8.00 Log_{10} IU/㎖ in Human serum. Both were within the HLoQ and LLoQ in Human serum. We claimed that *AccuPower®* HCV Quantitative RT-PCR Kit is possible to measure within range of 8.00 to 1.30 Log_{10} IU/㎖ in EDTA-plasma and Human serum.

It was included in the total analytical error (TAE) reference value of 1.00 Log_{10} IU/㎖. Therefore, claimed LOQ for *AccuPower®* HCV Quantitative RT-PCR Kit for all HCV Genotypes is between the 1.00 to 1.70 Log_{10} IU/㎖.

Table 10. Linear equation and range of all HCV genotypes analyzed

Matrix	HCV Genotype	Linear equation in genotype linearity study	Maximum difference between HCV genotype 1a and corresponding HCV genotype (Log_{10} IU/㎖)
EDTA-plasma	1a	y = 0.9385x + 0.0373	N/A
	1b	y= 1.1189x – 0.1718	0.10
	2a	y = 0.9649x – 0.068	0.06
	2b	y = 1.0264x –0.2885	0.18
	3	y = 1.0409x – 0.3147	0.18
	4	y = 1.1671x –0.3034	0.05
	5	y = 1.0234x – 0.2866	0.18
	6	y = 1.0706x – 0.3082	0.12
Serum	1a	y = 1.0071x – 0.0244	N/A
	1b	y =1.0127x + 0.0841	–0.13
	2a	y = 1.0051x + 0.0136	–0.03
	2b	y = 1.0744x – 0.3382	0.11
	3	y = 0.9636x + 0.0465	0.06
	4	y = 1.0582x – 0.1291	–0.05
	5	y = 1.2203x – 0.63	–0.03
	6	y = 0.9315x + 0.2427	–0.04

Table 11. LOQ of all HCV genotypes analyzed (Log_{10} IU/㎖)

HCV Genotype	Matrix	Nominal concentration	N	Average Measured Concentration	Bias	SD	TAE = |Bias| +2 x SD	SQRT[2]x2 x SD
1a	Plasma	1.18	40	1.15	0.0	0.31	0.65	0.88
	Serum	1.30	40	1.52	0.2	0.29	0.81	0.82
1b	Plasma	1.00	26	0.77	–0.2	0.27	0.77	0.77
	Serum	1.00	26	1.03	0.0	0.21	0.45	0.60
2a	Plasma	1.18	26	1.10	–0.1	0.19	0.45	0.53
	Serum	1.70	26	1.74	0.0	0.15	0.35	0.43
2b	Plasma	1.70	26	1.45	–0.3	0.14	0.52	0.39
	Serum	1.40	26	1.15	–0.3	0.19	0.64	0.54
3	Plasma	1.18	26	0.91	–0.3	0.30	0.86	0.84
	Serum	1.40	26	1.47	0.1	0.21	0.49	0.60
4	Plasma	1.18	26	1.06	–0.1	0.23	0.59	0.66
	Serum	1.18	26	1.10	–0.1	0.33	0.75	0.95
5	Plasma	1.70	26	1.44	–0.3	0.13	0.52	0.36
	Serum	1.70	26	1.37	–0.3	0.25	0.83	0.71
6	Plasma	1.40	26	1.20	–0.2	0.18	0.56	0.52
	Serum	1.70	26	1.89	0.2	0.17	0.53	0.48

9.1.5 Precision

The Precision study was performed to determine the Repeatability and Reproducibility of *AccuPower*® HCV Quantitative RT-PCR Kit. Test was performed using WHO 5th HCV International Standard panel (NIBSC code: 14/150, UK) containing 5.00 Log_{10} IU/㎖ of HCV genotype 1a.

9.1.5.1 Repeatability

For this test, three (3) serial dilutions of 5th HCV International Standard panel (NIBSC code: 14/150, UK) were prepared in EDTA-plasma (Seracare, Milford, USA) and Normal Human serum (Merck Millipore, Germany). Each panel consisted of three (3) HCV RNA concentrations of 3.00, 2.00, 1.48 Log_{10}IU/㎖ in plasma and 3.00, 2.00, 1.65 Log_{10} IU/㎖ in Human serum.

The evaluation was performed with three (3) different lots of *AccuPower*® HCV Quantitative RT-PCR Kit. Test was performed in three (3) different *ExiStation*™ systems. Each concentration was tested in two (2) replicates and two (2) runs per day, total twenty (20) day test. The results are summarized in table 12.

Table 12. The summary result of repeatability

Matrix	Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid tests	Within-Run (S_r)	Between-Run (S_{rr})	Between-Day (S_{dd})	Total precision (S_T)
EDTA-plasma	3.00	2.89	80	0.15	0.10	0.09	0.20
	2.00	1.82	80	0.23	0.12	0.10	0.27
	1.48	1.34	80	0.31	0.11	0.11	0.35
Serum	3.00	3.01	80	0.17	0.05	0.12	0.21
	2.00	1.92	80	0.18	0.03	0.12	0.22
	1.65	1.59	80	0.23	0.10	0.18	0.30

Repeatability results were within-run (S_r); 0.31, Between-run (S_{rr}); 0.12, Between-day (S_{dd}); 0.11, and Total precision (S_T); 0.35 in Plasma and within-run (S_r); 0.23, Between-run (S_{rr}); 0.10, Between-day (S_{dd}); 0.18, and Total precision (S_T); 0.30 in Serum.

9.1.5.2 Reproducibility

To determine reproducibility, three (3) serial dilutions of 5th HCV International Standard panel (NIBSC code: 14/150, UK) in EDTA-plasma (Seracare, Milford, USA) and Normal Human serum (Merck Millipore, Germany). Each panel and particles consisted of three (3) HCV RNA concentrations of 3.00, 2.00, 1.48 Log_{10} IU/㎖ in plasma and 3.00, 2.00, 1.65 Log10 IU/㎖ in serum. Each panel was evaluated in two (2) replicates, two (2) runs per day and over the period of five (5) days.

Between-Lot experiment was conducted in three (3) lots, two (2) replicates per sample, two (2) runs per day for five (5) days. The between-operator and between-instrument experiments

were conducted in a single lot, two (2) replicates per samples, two (2) runs per day for ten (10) days. The between-site experiment was conducted in a single lot, two (2) replicates per sample, two (2) runs per day for ten (10) days.
The evaluation was performed with three (3) different *Existation*™ system. Two different lots of *Exiprep*™ Dx viral DNA/RNA Kits and three (3) different lots of *AccuPower*® HCV Quantitative RT-PCR Kit were used for the study. The results are summarized in table 13.

Table 13. The summary result of reproducibility

Matrix	Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid tests	Standard Deviation(SD)			
				Between-Lot	Between-Site	Between-Operator	Between-Instrument
EDTA-plasma	3.00	3.04	140	0.19	0.25	0.21	0.25
	2.00	2.04	140	0.21	0.39	0.24	0.41
	1.48	1.52	140	0.33	0.37	0.34	0.42
Serum	3.00	3.14	140	0.23	0.25	0.18	0.24
	2.00	2.13	140	0.23	0.38	0.22	0.44
	1.65	1.78	140	0.28	0.39	0.24	0.34

9.1.6 Interfering substances

Twenty-two (22) exogenous substances (including anti-viral substance) and six (6) endogenous substances was used for interfering test of *AccuPower*® HCV Quantitative RT-PCR kit.
Potentially interfering endogenous and exogenous substances were spiked into EDTA plasma and serum in the absence or presence of three times LODx3 (Plasma 1.48 Log_{10} IU/㎖ and Serum 1.65 Log_{10} IU/㎖). HCV concentration of 3.00 Log_{10} IU/㎖ was compared to control EDTA plasma and serum without interfering substances. Each interfering substance was tested at concentration of 3.00 Log_{10} IU/㎖ and 1.48, 1.65 Log_{10} IU/㎖ in three (3) and fourteen (14) replicates.
All tested interfering substance showed no influence on the performance of *AccuPower*® HCV Quantitative RT-PCR Kit.

Table 14. Interference-Exogenous Interfering Substances

No.	Potential interfering substance	Concentration (ug/㎖)	No.	Potential interfering substance	Concentration (ug/㎖)
1	Entecavir	164ng/ml	**12**	Efavirenz	81.4ug/mL
2	Raltegravir	25.8mg/L	**13**	Ribavirin	54.96ug/L
3	Adefovirdipivoxil.	368ng/L	**14**	Trimethoprim	2760umol/L
4	Isoniazid	5840umol/l	**15**	Nelfinavir	40ug/mL
5	Lamivudine	44.8ug/ml	**16**	Nevirapine	40ug/L
6	pyrazina	576ug/ml	**17**	Ritonavir	224ug/L
7	Rifabutin	9200ng/ml	**18**	Saquinavir	104.16ug/mL
8	Rifampicin	312mg/l	**19**	Tenofovir	6ug/ml
9	Sulfameth	31.6mmol/l	**20**	Abacavir	60ug/L
10	Telbivudine	74ug/ml	**21**	Valganciclovir	113ug/mL

11	Amprenavir	153.2ug/mL	22	Zidovudine	45.8ug/ml

Table 15. Interference- Endogenous Interfering Substances

No.	Potential interfering substance	Concentration	No.	Potential interfering substance	Concentration
1	EDTA	540mg/dL	4	Hemoglobin	200mg/dL
2	Citrate	0.327M	5	Albumin	5g/dL
3	Heparin	3KU/dL	6	Bilirubin	25mg/dL

9.1.6 Cross reactivity

The following viruses and bacteria were tested for cross-reactivity of *AccuPower*® HCV Quantitative RT-PCR Kit. Samples were prepared by diluting organisms or DNA/RNA either in following matrix. HCV negative EDTA-plasma/serum or HCV spiked EDTA plasma/serum at 3x LoD (EDTA-plasma: 1.48 Log_{10} IU/㎖, serum: 1.65 Log_{10} IU/㎖). Test was performed in three (3) replicates.

Negative EDTA-plasma and serum showed negative detection. HCV positive specimens spiked in cross-reactivity organisms showed detection at ±0.50 Log_{10} IU/㎖ and ±0.50 Log_{10} IU/㎖ in plasma and serum respectively.

Table 16. List of potential cross reactivity organism

Viruses		Bacteria
Hepatitis A virus	Varicella-Zoster Virus	*Neisseria gonorrhoeae*
Hepatitis B virus	West Nile Virus	*Chlamydia trachomatis*
HIV-1	Zika Virus	*Mycobacterium gordonae*
HIV-2	Human herpesvirus 6B	*Staphylococcus aureus*
Epstein-Barr Virus	Human herpesvirus 8	
Cytomegalovirus	Adenovirus type 5	
Human papilloma virus 16	Dengue virus types 1	
Human papilloma virus 18	Dengue virus types 2	
BK human polyomavirus	Dengue virus types 3	
Herpes simplex virus 1	Dengue virus types 4	
Herpes simplex virus 2	Influenza Virus A(H1N1)	
Influenza Virus A(H3N2)	Powassan virus	
St. Louis encephalitis virus	Usutu virus	
Japanese encephalitis immune ascitic fluid		

9.1.7 Whole system failure

The Whole System Failure rate was tested with one-hundred two (102) replicates using the *AccuPower*® HCV Quantitative RT-PCR Kit. Positive results were obtained 100% detection of the one-hundred two (102) replicates overall, a system success rate was shown 100% in the *AccuPower*® HCV Quantitative RT-PCR Kit.

Table 17. Whole system failure results

Matrix	Concentration (Log_{10} IU/㎖)	Test number	Detection rate(%)
Plasma	1.48	102	100%
Serum	1.65	102	100%

.1.8 Cross contamination

This evaluation consists of eight (8) samples each of High positive and Negative, and five (5) runs on the same instrument for 5 days. All negative sample should not be detect a HCV signal. The cross-contamination test was performed by using the HCV diagnostic kit according to the CTS guideline. High positive and negative were tested at HLoQ concentration(8.00 log_{10} IU/㎖) and Negative HCV free matrix, respectively.

Table 18. Cross contamination results in EDTA-plasma

Run	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	Negative	8.00	100%
Run1	8/8	0/8	7.83	Not detected
Run2	8/8	0/8	7.77	Not detected
Run3	8/8	0/8	7.80	Not detected
Run4	8/8	0/8	7.86	Not detected
Run5	8/8	0/8	7.86	Not detected
Average			7.83	-
SD			0.04	-

Table 19. Summary of cross-contamination results (Between equipment_Plasma)

Equipment	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	Negative	8.00	100%
Equipment 1	8/8	0/8	8.02	Not detected
Equipment 2	8/8	0/8	8.03	Not detected
Equipment 3	8/8	0/8	8.05	Not detected
Equipment 4	8/8	0/8	8.05	Not detected
Equipment 5	8/8	0/8	8.02	Not detected
Average			8.03	-
SD			0.04	-

Table 20. Cross contamination results in Serum

Run	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	Negative	8.00	negative
Run1	8/8	0/8	7.89	Not detected
Run2	8/8	0/8	7.85	Not detected
Run3	8/8	0/8	7.92	Not detected
Run4	8/8	0/8	7.87	Not detected
Run5	8/8	0/8	7.87	Not detected
Average			7.88	-
SD			0.058	-

Table 21. Summary of cross-contamination results (Between equipment_Serum)

Equipment	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	Negative	8.00	negative
Equipment 1	8/8	0/8	8.03	Not detected
Equipment 2	8/8	0/8	8.02	Not detected
Equipment 3	8/8	0/8	8.05	Not detected
Equipment 4	8/8	0/8	8.08	Not detected
Equipment 5	8/8	0/8	7.98	Not detected
Average			8.03	-
SD			0.07	-

9.2 Diagnostic Performance Characteristics

9.2.1 Diagnostic Sensitivity & Specificity

Total of two-hundred fifty (250) HCV positive and Negative EDTA plasma clinical sample were compared with CE-IVD approved HCV NAT assay.
Diagnostic sensitivity was 99.26% (95% CI 95.95 – 99.87) and the specificity was 99.12 % (95% CI 95.21 – 99.84). This satisfies the proposed acceptance criteria of 95% or more.

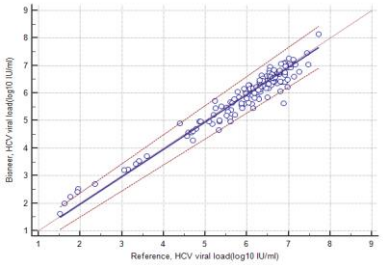
Table 22. HCV Clinical evaluation results summary of *AccuPower*® HCV Quantitative RT-PCR Kit.

ExiStation™ system	CE-IVD approved HCV NAT assay		
	Positive	Negative	Total
Positive	135	1	136
Negative	1	113	114
Total	136	114	250

Diagnostic Sensitivity (Percent positive agreement) = 99.26 % (95% C.I 92.95 – 99.87)
Diagnostic Specificity (Percent negative agreement) = 99.12 % (95% C.I 95.21 – 99.84)

9.2.2 Correlation

AccuPower® HCV Quantitative RT-PCR Kit was compared with other CE-IVD approved HCV assay.
Total of 136 specimens were collected from HCV infected patients and tested at external site. 135 of 136 results were valid. Result was analyzed with linear regression method.
The R^2 value was 0.9435, the slope was 0.9939(95% CI: 0.9470 ~ 1.0559) and the intercept was −0.007(95% CI: −0.3942~0.2695)Log_{10} IU/㎖.



Regression Equation	y = -0.007 + 0.9939 x
Intercept	-0.007(95% CI: -0.3942~0.2695)
Slope	0.9939(95% CI: 0.9470 ~ 1.0559)
R^2	0.9435
Linear model validity (Cusum test for linearity)	P=0.31 (P> 0.05)

Fig 74. Correlation with CE-IVD approved assay.

9.2.3 Verification of precision

Precision of the kit was validated by manufacturer. Results of manufacturer`s precision test was verified in clinical site. This study was analyzed using two (2) dilution of HCV international standard panel and a single lot of *AccuPower*® HCV Quantitative RT-PCR Kit according to CLSI EP15-A. Each dilution was tested in three (3) replicates and over the period of three (3) days. Result of user's precision test was lower than manufacturer's precision test.

The S_{within} or S_{Total} precision of *AccuPower*® HCV Quantitative RT-PCR Kit assay was verified to be consistent with manufacturer's claim.

Table 23. Summary results of User`s precision verification

	Analytical performance Precision value		Verification performance Precision value		Verification performance verification value	
	σ_{within}	σ_{total}	S_{within}	S_{total}	S_{within}	S_{total}
30 IU/㎖	0.31	0.35	0.43	0.41	0.48	0.49
1,000 IU/㎖	0.15	0.2	0.16	0.15	0.23	0.29

10. TROUBLESHOOTING

Comments and suggestions	
Internal Positive Control (IPC) invalid results	
If the TAMRA (IPC) Fluorescence signal was not detected in all wells (including controls)	• Extraction and/or PCR configuration error ☞ Make sure that the correct extraction/PCR protocol was programmed and performed in accordance with the Kits. Repeat the assay, if necessary. See *User's Guide* 8. PROTOCOL • Incorrect extraction or PCR kit use ☞ Make sure that you use proper kits for the intended tests. • The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See *User's Guide* 5. STORAGE CONDITION AND SHELF • Invalid results. ☞ It must be tested with the new reagent
If the TAMRA (IPC) Fluorescence signal was not detected in particular wells.	• Inhibition of PCR ☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition. ☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type. • Low elution volume due to insoluble material of samples ☞ Yield of nucleic acid can be affected by sample conditions (viscosity etc.). Repeat the assay from the sample pretreatment process which can make the sample more soluble.

SPC/PC invalid results	
If the FAM (SPC) Fluorescence signal was undetermined.	• The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See *User's Guide* 5. STORAGE CONDITION AND SHELF LIFE • Re-use of reagents ☞ Make sure not to re-use reagents. Re-use or repeated freeze/thaw cycles of reagents may affect the kit quality and the results of assay conclusively. Repeat the assay with new reagents, if necessary. See *User's Guide 5*. STORAGE CONDITION AND SHELF General Precautions • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm the amount of SPC used in a single well. See *User's Guide* 8. PROTOCOL • There may have been a pipetting error. ☞ Review the pipetting technique and calibration. • Invalid results. ☞ It must be tested with the new reagent
No template Control (NTC) invalid results	
If the FAM fluorescence signal was detected in NTC well.	• Contamination may have occurred. ☞ Make sure that work space and instruments are decontaminated and repeat the assay. • The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See *User's Guide* 5. STORAGE CONDITION AND SHELF • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm whether controls and samples are loaded in proper wells which are assigned through S/W protocol (especially NTC well(s)). See *User's Guide* 8. PROTOCOL • There may have been a pipetting error. ☞ Review the pipetting technique and calibration.

11. REFERENCES

Mackay IM. (2004) Real-time PCR in the microbiology laboratory. Clin.Microbiol. Infect. 10:190-212

EASL Recommendations on Treatment of Hepatitis C, 2018

Hepatitis C Virus Genotype 7, a New Genotype Originating from Central Africa, Journal of Clinical Microbiology, March 2015 Volume 53 Number 3.

MESSINA ET AL. Global Distribution and Prevalence of Hepatitis C virus Genotypes, HEPATOLOGY, January 2015.

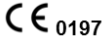
Lavanchy, Evolving epidemiology of Hepatitis C virus, Clinical Microbiology and Infection, Volume 17 Number 2, February 2011

Philippe H. (2006) Real-Time PCR Assay for Hepatitis C virus (HCV) RNA Quantification Are Adepuate for Clinical Management of Patients with Chronic HCV Infection. Journal of Clin.Microbiol. 44:2507-2511

Fernanda M. (2007) Comparison of Performance Characteristics of Three Real-Time Reverse Transcription-PCR Test Systems for Detection and Quantification of Hepatitis C virus. Journal of Clin. Microbiol.45:2529-2536

.

12. SYMBOLS

	Catalog number		Temperature limitation
	In vitro diagnostic medical device		Contains sufficient for test
	Manufacturer		Caution, consult accompanying documents
	Batch code		Expiration date
	Do not reuse		Consult instructions for use
	Warning for hazardous and irritation		Keep away from sunlight
	Conformite Europeenne Mark		Authorized representative in the European Community

Bioneer Worldwide

Bioneer Corporation

Address 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
Tel +82-42-930-8777 (Korea: 1588-9788)
Fax +82-42-930-8688
E-mail sales@bioneer.com
Web www.bioneer.com

Bioneer Inc.

Address 155 Filbert St. Suite 216 Oakland, CA 94607, USA
Tel +1-877-264-4300 (Toll-free)
Fax +1-510-865-0350
E-mail order.usa@bioneer.com
Web us.bioneer.com

Bioneer R&D Center

Address Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si Gyeonggi-do, 13488, Republic of Korea
Tel +82-31-628-0500
Fax +82-31-628-0555
E-mail sales@bioneer.co.kr
Web www.bioneer.co.kr