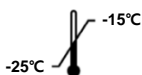


# USER'S GUIDE

CE 0197

Innovation • Value • Discovery

## AccuPower® HBV Quantitative PCR Kit



**REF**

**HBV-1211**

**IVD**

Quantitative test kit  
Hepatitis B virus DNA

**EC**

**REP**

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# AccuPower® HBV Quantitative PCR Kit

## User's Guide



Version No.: 4.4 (2021-09-09)

**Please read all the information in booklet before using the unit**



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## 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.

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## 1. INTENDED USE

*AccuPower®* HBV Quantitative PCR Kit is an in vitro diagnostic kit designed for quantification of HBV (Hepatitis B Virus) DNA in human EDTA-plasma samples or human serum through real-time polymerase chain reaction (PCR) using *ExiStation™* Universal MDx system. *AccuPower®* HBV Quantitative PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for monitoring of patient's prognosis or treatment by measuring HBV viral load. The linearity of *AccuPower®* HBV Quantitative PCR Kit was 1.18 ~ 8.00 Log<sub>10</sub> IU/mL and the linearity of HBV was equal to linearity of each genotype (A, B, C, D, E, F, G and H). This kit is not intended to be used as a screening test for HBV infection in clinical samples including blood and blood products. It is not intended for initial clinical diagnosis of HBV infection, like a HBV screening.

## 2. INTRODUCTION

HBV is one of the smallest viruses known to infect humans, and belongs to the hepadnavirus family. It is a hepatotropic virus, and liver injury occurs through immune-mediated killing of infected liver cells. All patients with chronic HBV infection are at increased risk of progression to cirrhosis and hepatocellular carcinoma (HCC), depending on host and viral factors. The main goal of therapy is to improve survival and quality of life by preventing disease progression, and consequently HCC development. Ten genotypes of hepatitis B virus (A-J) are currently recognized, The genotypes show a distinct geographical distribution between and even within regions, and are proving to be an invaluable tool in tracing the molecular evolution and patterns and modes of spread of hepatitis B virus

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood and various body fluids, including saliva, menstrual, vaginal, and seminal fluids, which have all been implicated as vehicles of human transmission. Infection in adulthood leads to chronic hepatitis in less than 5% of cases. Transmission of the virus may also result from accidental inoculation of minute amounts of blood or fluid during medical, surgical and dental procedures, or from razors and similar objects contaminated with infected blood; use of inadequately sterilized syringes and needles; intravenous and percutaneous drug abuse; tattooing; body piercing; and acupuncture.

The most common marker for detecting a HBV infection is characterized by the presence of antibodies (anti-HBs and anti-HBc) and HBeAg is used as a secondary marker to indicate active HBV replication associated with progressive liver disease. HBV DNA concentrations quantified by real-time polymerase chain reaction (PCR) correlate with disease progression and are very useful to evaluate the efficacy of antiviral therapy.

*AccuPower®* HBV Quantitative PCR Kit allow detection of HBV genotype A-H and confirmed that can detect a HBV genotype I and J through In-silico analysis. with vacuum-drying, increase a product stability and BIONEER's own Mdx system from nucleic acid extraction to qPCR.

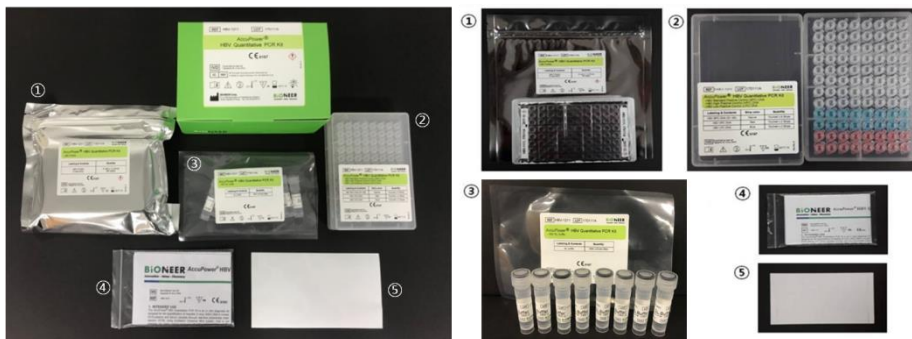
### **3. FEATURES AND PRINCIPLE OF THE TEST**

Real-time PCR involves the selective amplification of a target sequence (HBV surface antigen) while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. 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



Contents of AccuPower® HBV Quantitative PCR Kit

No.	Reagent	Unit	Quantity	Function
①	HBV PCR Premix	8-well/strip X 12 strips(96 tests) (in aluminum foil bag)	1 pack	NA amplification
②	HBV SPC²DNA 1 (S1) ; 2 X 10² copies/μℓ	15 μℓ / well (Natural 8-tube strip)	8 strips	Calibration
	HBV SPC²DNA 2 (S2) ; 2 X 10³ copies/μℓ			
	HBV SPC²DNA 3 (S3) ; 2 X 10⁴ copies/μℓ			
	HBV SPC²DNA 4 (S4) ; 2 X 10⁵ copies/μℓ			
	HBV SPC²DNA 5 (S5) ; 2 X 10⁶ copies/μℓ			
	HBV LPC²; 2 X 10² copies/μℓ	15 μℓ / well (Blue 8-tube strip)	2 strips	Positive Control
	HBV HPC²; 2 X 10⁴ copies/μℓ	15 μℓ / well (Red 8-tube strip)	2 strips	
③	SL buffer	1800 μℓ / tube(Clear 2mℓ screw tube)	8 tubes	Control loading buffer (for NTC¹, SPCs, PCs), Sample dilution
④	Quick Manual	-	1 ea	
⑤	Optical sealing film	-	1 ea	Sealing of PCR Premix
⑥	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. For detailed instructions, see 8. Protocol in this User's Guide.

## 5. STORAGE CONDITION AND SHELF LIFE



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

## 6. REQUIRED MATERIALS AND EQUIPMENT (NOT PROVIDED IN THE KIT)

System	Instrument	Reagent (Extraction)
<i>ExiStation™</i> (A-2200)	- <i>ExiPrep™</i> 16 Dx (Cat. No. A-5050) - <i>Exicycler™</i> 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)	- <i>ExiPrep™</i> Dx Viral DNA Kit (K-4472) - <i>ExiPrep™</i> Dx Viral DNA/RNA Kit (K-4471) - Sample Loading Tube_DNA IPC (Cat. No. KA-3010)
	<i>ExiStation™</i> manager software (Version 1.02. XX)	
<i>ExiStation™</i> (A-2200-N)	- <i>ExiPrep™</i> 16 Dx (Cat. No. A-5050) - <i>Exicycler™</i> 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1)	- <i>ExiPrep™</i> Dx Viral DNA Kit (K-4472) - <i>ExiPrep™</i> Dx Viral DNA/RNA Kit (K-4471) - Sample Loading Tube_DNA IPC (Cat. No. KA-3010)
	<i>ExiStation™</i> manager software (Version 4.02. XX)	
<i>ExiStation™</i> 48 (A-2400) <i>ExiStation™</i> 48A (A-2410)	- <i>ExiPrep™</i> 48 Dx (Cat. No. A-5150) - <i>Exicycler™</i> 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1) - <i>ExiLT</i> (Cat.No. A-7100)	- <i>ExiPrep™</i> 48 Viral DNA/RNA Kit (K-4571) - <i>Exiprep™</i> 48 Sample Loading Tube_IPC (KA-4501)
	<i>Exiprep™</i> 48 software (Version 1.0.X.X)	
Etc	- <i>ExiSpin™</i> (Cat.No. A-7040)	N/A
General lab- equipment and disposables	<ul style="list-style-type: none"> <li>• Disposable powder-free gloves</li> <li>• Pipette set appropriate volume (1,000 <math>\mu</math>l, 200 <math>\mu</math>l, 20 <math>\mu</math>l pipette)</li> <li>• Sterilized pipette tips with filters(1,000 <math>\mu</math>l, 200 <math>\mu</math>l, 20 <math>\mu</math>l tips with filters)</li> <li>• 1.5 ml or 15 ml conical tubes</li> </ul>	



## 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 user and the laboratory safety and the prevention of laboratory environmental contamination.

When handling clinical samples, all related work (de-capping, pipetting, and capping of clinical samples and containers) **should be conducted within a negative pressure biosafety cabinet (class II or III)**. A negative pressure biosafety cabinet sends air from the laboratory space to outside. In other words, air flows inwards. 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. A Cleanbench is a workspace where filtered air flows outwards, thus keeping a clean environment within the workspace.

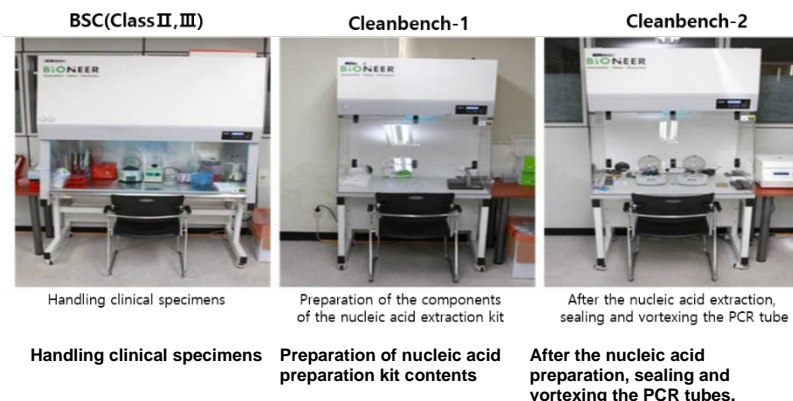


Fig. 1 Biosafety Cabinet (BSC) & Cleanbench

## 8.2 Specimen



All sample should be treated as potential biohazards. For the best results, DNA extracted from human EDTA-plasma samples or from human serum is recommended.

### 8.2.1 Specimen Collection

The AccuPower® HBV Quantitative PCR Kit is optimized for DNA extracted from human EDTA-plasma samples or from human serum. For EDTA-plasma collection or for human serum, 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 EDTA tubes should be stored and/or transported for up to 24 hours at 2°C to 30°C.

### 8.2.3 Specimen Storage

The isolated human EDTA-plasma or human serum can be stored up to 6 days between 2°C and 8 °C. For long period of storage, samples should be stored at following condition. Room temperature (between 25°C and 30°C) for up to 1 day or Temperature between 2°C and 8°C for up to 6 days, between -70°C and -80°C for 6 months.

### 8.2.4 Interfering Substances

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

For the optimal PCR results, the interference materials in the specimens would be eliminated during the DNA extraction process using the ExiStation™ Universal Molecular Diagnostic System.

### 8.3 Work Flow

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

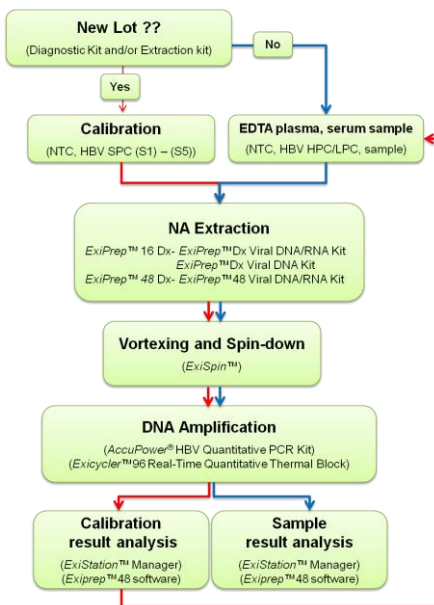


Fig. 2 Work flow

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

## 8.4 Experimental Procedure | (*ExiStation*™ system)

### Part 1. Assigning test using *ExiStation*™ Manager software

\* 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 DNA Kit (K-4472). 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) Press the main power button, as shown in Fig 4 below, to initiate *ExiPrep*™16 Dx. Once the starting button appears on the LED display, tap the button to 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) Load the Tip Protector and the Contamination Shield with filter paper equipped in the equipment. Tap the "MISC SET" button again to set the syringe block in place.

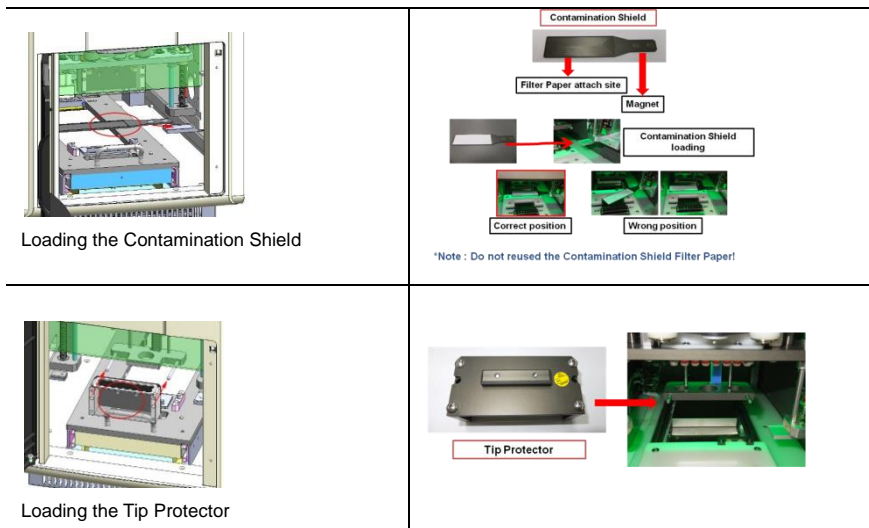


Fig. 6 Loading the Contamination Shield, Tip Protector

- 6) Close the door once the tray is all set. Tap "UV lamp" button on the display to initiate UV sterilization. The sterilization will automatically run for 15 minutes.
- 7) *ExiStation™* Manager Software has 6 distinct part.

**Prep** - controls nucleic acid extraction (*ExiPrep™* 16 Dx instrument),

**Assign PCR** - transfer sample information from "Prep" to "PCR" (*Exicycler™* 96) and assigns for PCR run

**PCR** - displays real-time amplification conditions (*Exicycler™* 96)

**Result** - presents the results, experiment information, and the sample information after the PCR process has been completed

**Configuration** - software setup information (accessible only by the manufacturer)

**Version** - displays software version



Fig. 7 Main screen of *ExiStation™* Manager software

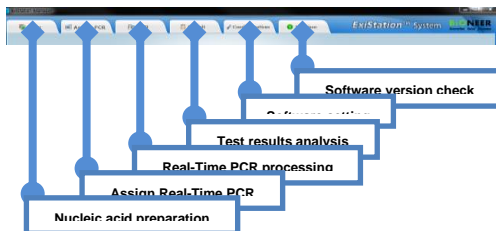


Fig. 8 Tab function of ExiStation™ Manager software

- 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

The prep control panel consists of 5 panels.

**Instruments status panel** – displays the status of ExiPrep™16 Dx

**Kit selection panel** – allows selection/input of the diagnostic kit, prep kit, and lot information (optional: barcode scanning system)

**Sample and control information panel** – allows input of control (NTC, PC, SPC) and sample information (optional: barcode scanning system)

**Well information panel** – displays the well information with different colors

**ExiPrep™16 Dx control panel** – controls ExiPrep™ 16 Dx including UV controller, Store controller, Running controller, and MISC set controller

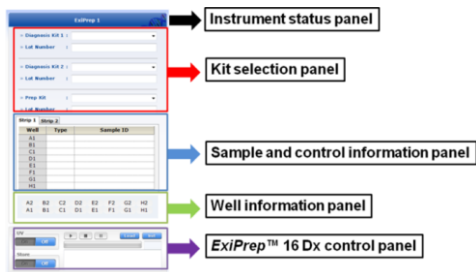


Fig. 10 Prep control panel of ExiStation™ Manager software

- 9) Click the pull-down arrow for “Diagnosis Kit 1”. A popup window will appear. Select “HBV-1211” from the pull-down menus

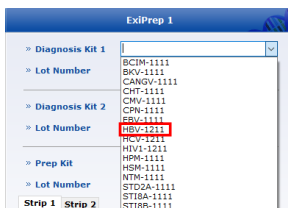


Fig. 11 Selection of Diagnostic kit

- 10) Once “Diagnosis Kit” is selected, sample allocation window will popup. Select any undesired Wells in the buffer cartridge and mark the locations as shown in figure below. Select the “OK” to finish.

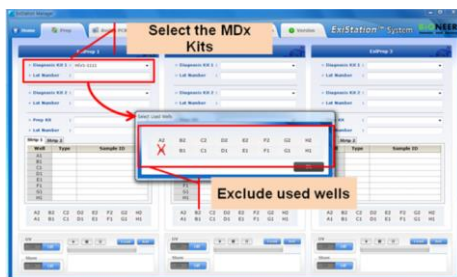


Fig. 12 'Prep' Pop-up window of ExiStation™ Manager software

- 11) Click the pull-down arrow for “Prep Kit”. A popup of the appropriate “Prep Kit” for the selected diagnostic kit will 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.
- 13) The lot of diagnostic kit and/or extraction kit is new, the program automatically assigns the NTC and SPC from 1 to 5. When the same lot combination of the diagnostic kit and extraction kit is used to the 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

- 14) Click the "Sample ID" column and enter sample information either typing in manually or using a barcode reader (optional).

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

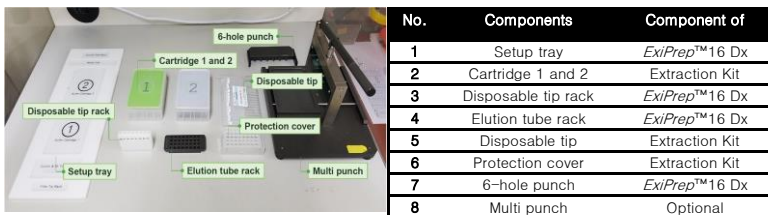
## Part 2. Nucleic acid extraction by *ExiPrep™ 16 Dx*

- 1) BIONEER recommends using the BSC (Class II) and clean bench for *ExiStation™* system operation.
- 2) Clean the surface (preferably aCleanbench-1) where work will be performed.

**⚠ Wipe the surface with 0.5% sodium hypochlorite and 70% ethanol or DI water to prevent contamination. After wiping, turn on the UV lamp to eliminate contaminants.**

**⚠ Turn off the UV lamp when using the BSC.**

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



**Fig. 15 List of necessary components for nucleic acid extraction**

- 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 well's bottom.**



**Fig. 16 Remove the acryl lids**

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

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

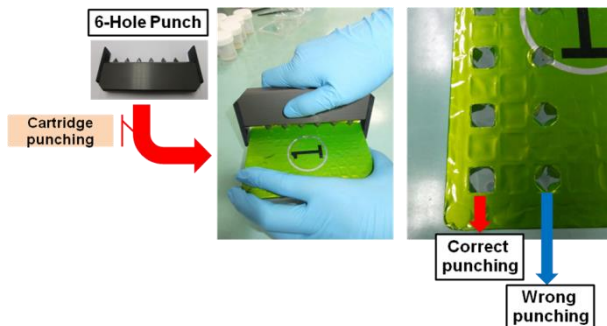


Fig. 17 Punch the film with the Hole Puncher

- 6) Cover Buffer Cartridges ① and ② with the lids after film punching is complete.
- 7) Place Buffer Cartridges on the setup tray.

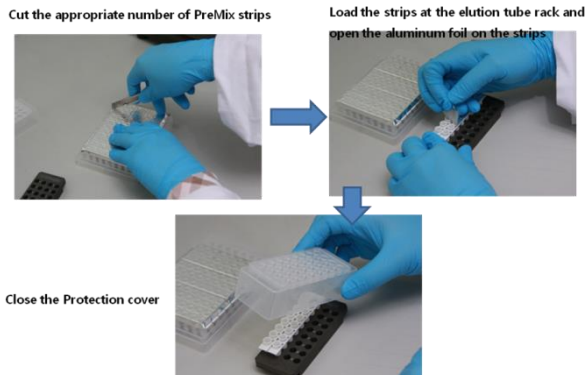


Fig. 18 Install buffer cartridge on the set-up tray

- 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. It is recommended to mark each strip of the diagnostic tubes with the corresponding column number.

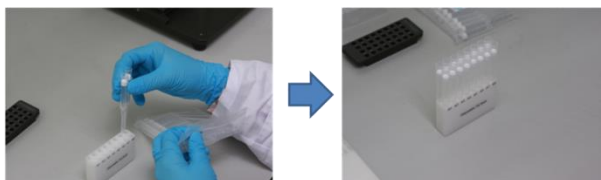
⚠ Ensure that the diagnostic tubes are marked so they can be identified later.

⚠ 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. 19** Load the AccuPower® Diagnostic Kit tubes at the Elution Tube Rack

- 9) 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. 20** 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. 21** Load the Elution Tube Rack on the Setup tray.

13) Move Setup tray to the *ExiPrep*™ 16 Dx. Open the door and pull the base plate of *ExiPrep*™ 16 Dx.

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 result in experiment failure or an instrument malfunction.

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 firmly press the cartridge's right-hand side.

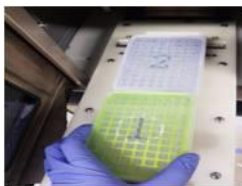


Fig. 22 Loading the Buffer Cartridge(2)

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

Note: Check the Protection Cover is adequately secured on the Elution Tube Rack.



Fig. 23 Inserting the Elution Tube Rack and Disposable Tip Rack

17) 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.

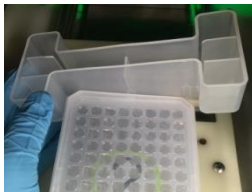


Fig. 24 Install of Waste Tray

- 18) Slide the Base Plate in and close the door of the *EixPrep*™ 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 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 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) Please take out the DNA IPC Sample Loading Tubes from the packaging, mark it with a sample name and insert them into the rack.



**Before using a Sample Loading Tube, the bottom of the Sample Loading Tube MUST BE check for Blue color (dried IPC for DNA)**

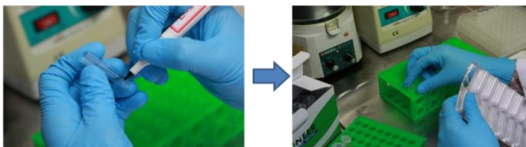


Fig. 26 Preparing Sample Loading Tube

- 21) Take the original clinical sample containers and controls (NTC and SPC) and pipette into the DNA IPC Sample Loading Tubes by following steps 22) to 25).
- 22) Add 400  $\mu\text{l}$  of SL buffer into a tube assigned as NTC. (supplied with the AccuPower® Diagnostic Kit)
- 23) Additionally add 395  $\mu\text{l}$  SL buffer and 5  $\mu\text{l}$  of SPC 1–5 into the appropriate SPC wells. (supplied with the AccuPower® Diagnostic Kit)

**When the assay is repeated with the same lot combination of Diagnostic kit and extraction kit, add 400  $\mu\text{l}$  of LPC (blue cap) and HPC (red cap) into each assigned well. calibration is Not needed this case.**

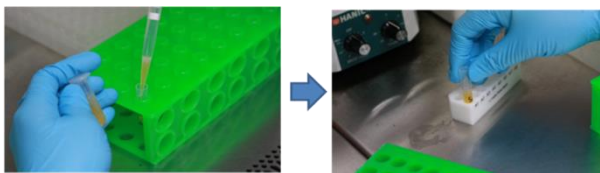
**NTC: Add SL buffer 400  $\mu\text{l}$  for NTC well.**

**LPC: Add 395  $\mu\text{l}$  SL buffer and 5  $\mu\text{l}$  of LPC (Blue) into LPC well.**

**HPC: Add 395  $\mu\text{l}$  SL buffer and 5  $\mu\text{l}$  of HPC (Red) into HPC well.**

- 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  $\mu\text{l}$  sample into DNA IPC Sample Loading Tube. Move the DNA 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.

**⚠ In case of any contamination of tips or gloves are suspected, immediately change gloves and tips to prevent the sample contamination.**

**⚠ DO NOT remain a DNA IPC Sample Loading Tube over 1 hours.**

- 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.**

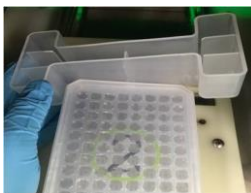


Fig. 30 Re-load Waste Tray

- 30) All materials are loaded.
- 31) Remove the lids from Buffer Cartridges.



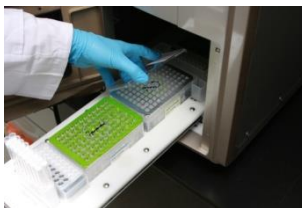


Fig. 31 Remove the lids

32) Check whether all accessories are loaded properly.



**Ensure the tips, holes and tubes are all in alignment.**

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



**Gently push the base plate in to prevent any sample or reagent spilling.**

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

- 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.

⚠ The nucleic acid extraction process takes 80~100 minutes according to the type of nucleic acid.

⚠ Suppose any error messages appear during the extraction process. Contact local BIONEER's distributor or headquarter for technical assistance.



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

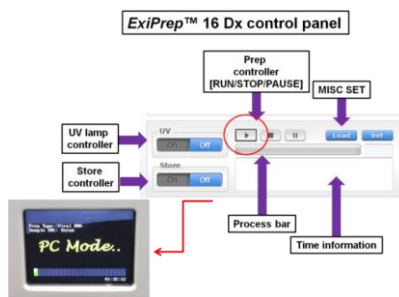


Fig. 33 *Exiprep*™ 16 Dx control panel

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



When sliding the base plate out, gently pull the base plate not to spill the waste.

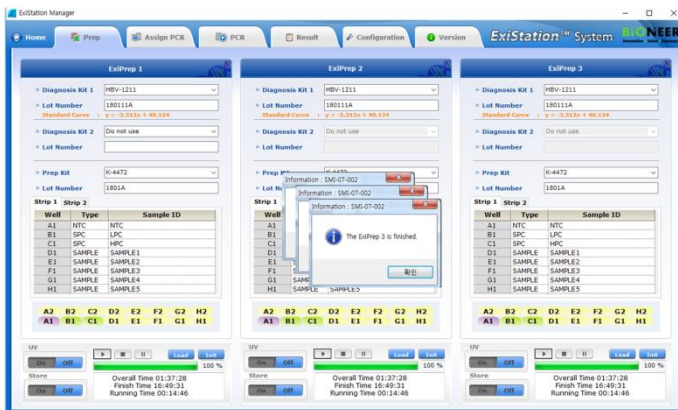


Fig. 34 Pop-up message for extraction finished

- 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, the 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 the Elution Tube Rack on Protection Cover Separation Tool, the lever must be facing the left-hand side.

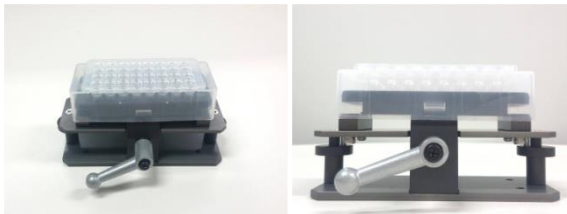


Fig. 36 Picture of Elution Tube Rack on top of Protection Cover Separation Tool

- 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.

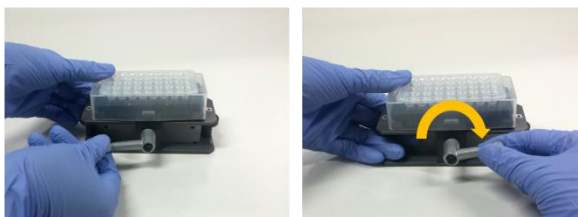


Fig. 37 Picture of lever rotation for fixing Elution Tube Rack to Protection Cover Separation Tool

- ③ Press both sides down of the Separation Tool shown 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 the Separation Tool consecutively to prevent any liquid from splashing.

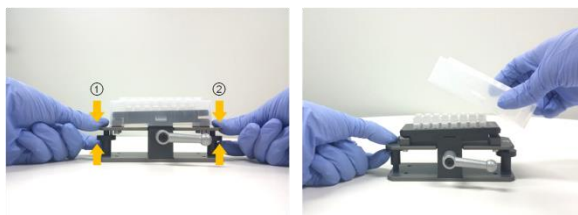


Fig. 38 Picture of pressing down each side of Separation Tool and removing Protection Cover from Separation Tool

- 5) Seal PCR Tube using Optical sealing film and then proceed to the next step. For more information on the 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 tube sat 4° C until use (if the prep reaction is divided into 2 steps, store it until 2<sup>nd</sup>prep finishes).**

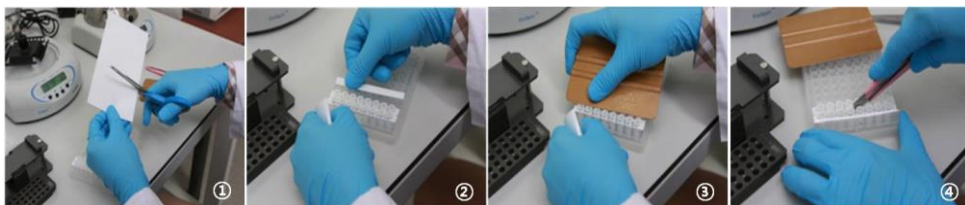


Fig. 39 Seal PCR premix strip

- 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.**
- ⚠ **Ensure to mark each diagnostic kit to prevent mix up.**
- ⚠ **When nucleic acid extraction is finished, the next step should be progressed within 10 minutes. If not, this may lead to an inaccurate result.**



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

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

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



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

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

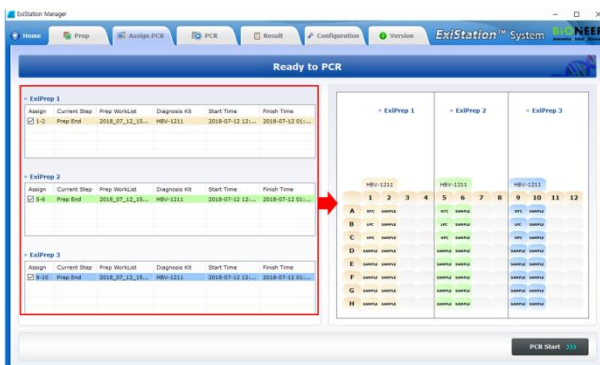


Fig. 42 'Assign PCR' tab - PCR Start

- 10) 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 completed, push the Door Switch for 2 seconds to close the door.

- ⚠ Ensure 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*<sup>™</sup> 96.

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

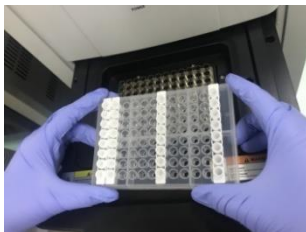


Fig. 43 Way to PCR Premix Strip setup of *Exicycler*™ 96

- 12) Select the 'Assign PCR' tab and confirm the 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 window's bottom right-hand side. 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'.

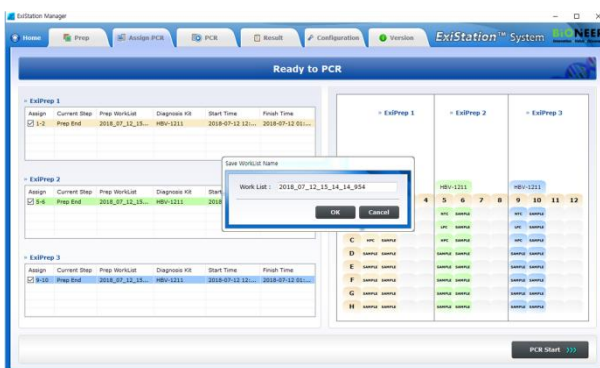


Fig. 44 Pop-up window of "Data name"

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

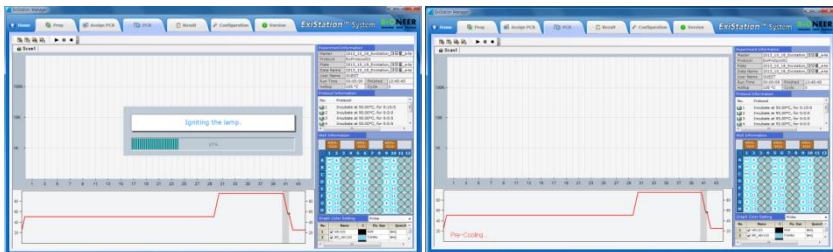


Fig. 45 PCR Running screen

- 14) 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 Cleanbench-1 for later use.
- ⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

- 15) Press the 'Misc Set' button, remove Tip Protector and Contamination Shield then press the 'Misc Set' button again
- 16) Push the Base Plate in, shut the instrument door and initiate UV sterilization by clicking "UV ON" on the control panel.

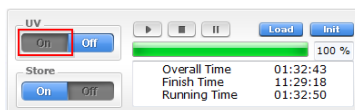


Fig. 46 *ExiPrep*™ 16 Dx control panel – UV



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

- ⚠ 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 procedures.

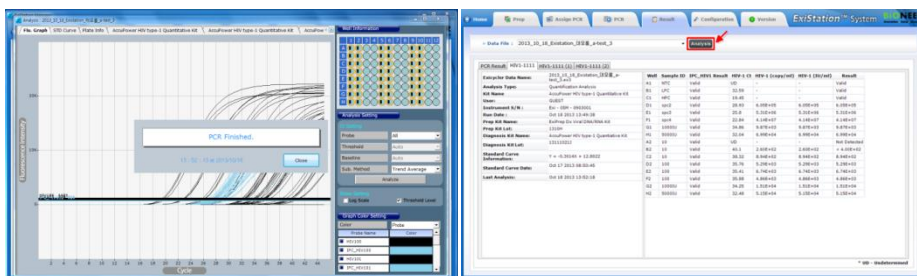


Fig. 47 Result analysis using ExiStations™ Manager software

- 18) 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 the *User's Guide of ExiPrep™ 48 Viral DNA/RNA Kit*, *ExiPrep™ 48 Dx* or *ExiLT™* for basic workflow.

- 1) Turn the *ExiPrep™ 48 Dx* switch on the back of the instrument, and press the POWER button on the front of the instrument for over 1 second.
- 2) As it starts to initialize, the LCD screen will automatically appear.
- 3) When the initialization of the instrument is completed, the main screen appears on the LCD screen. If initialization is NOT successfully completed, contact BIONEER or related agencies.



Fig. 48 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

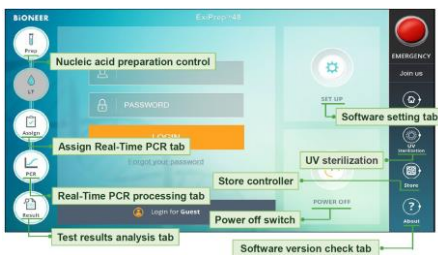


Fig. 49 Icons function of *ExiPrep™ 48* software

- 5) Log in with the registered ID. When logging in as a guest, it is usually saved data in the folder specified. Specify a folder efficient resulting data management.
- 6) Before the Prep, separate the Contamination Shield from ExiPrep™ 48 Dx. Clean with 70% EtOH, attach the Contamination Shield filter paper, and install the prepared Contamination Shield.



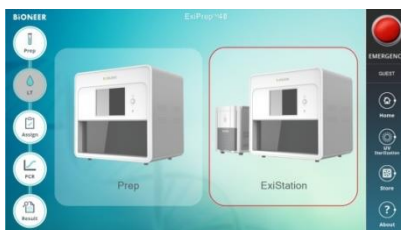
**Fig. 50 Decontamination step-1 ; Separation and installation of the Contamination Shield**

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



**Fig. 51 Decontamination step-2 ; UV sterilization**

- 8) Touch the Prep icon on the main screen, as shown below 52. Enter Prep mode for nucleic acid extraction by touching the ExiStation.



**Fig. 52 Initial screen of Prep tab**

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



Fig. 53 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. When there is no used wells to select, straight proceed to next step by clicking the “OK” button.

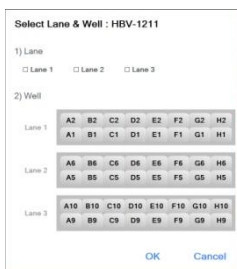


Fig. 54 Screen of 'Select Lane & Well'

- 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. 55 Entering Kit information

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

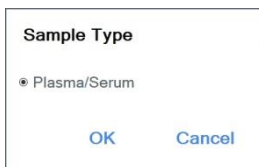


Fig. 56 Screen of ‘Sample Type’

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

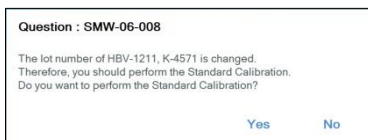


Fig. 57 Pop-up message for Standard Calibration process



- 15) The positions of NTC and SPC are automatically displayed in the Buffer Cartridge's remaining wells, and the default setting is set to 1 each of NTC and SPC 1-5. If the experiment is repeated with a combination of extraction/diagnosis kits of the same lot, since the automatically saved standard curve is used, LPC (Low Positive Control) and HPC (High Positive Control) is set for 1 well instead of SPC 1-5.
- 16) Complete generate of Standard curve normally, proceed the next experiment using clinical samples. If the experiment works, several information appears; standard curve, NTC/LPC/HPC's right position. Then click the 'Sample ID', input the clinical sample's information. (Optional-using barcode reader).



Fig. 58 Entering sample information

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


- 1) It is recommended that handling clinical samples and all related works should be conducted within a negative pressure BSC (Class II) for user safety and prevention of contamination.
- 2) Clean the BSC and check all necessary components for extraction and sample before nucleic acid extraction. Prepare extraction components within a Cleanbench-1. 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 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 the operation within Clean bench-1.

Prep tools	Consumables
<ul style="list-style-type: none"> <li>■ Setup Tray</li> <li>■ Hole Punch</li> <li>■ Sample Tube Rack</li> <li>■ Elution Tube Rack</li> <li>■ Clamp</li> </ul>	<ul style="list-style-type: none"> <li>■ Buffer Cartridges ① and ②</li> <li>■ Sample Loading Tubes_IPC</li> <li>■ Disposable Tips &amp; Rack</li> <li>■ Elution Tubes</li> <li>■ Elution Tube Caps</li> <li>■ Waste Tray</li> <li>■ Contamination Shield Filter Paper</li> </ul>

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

-  **Ensure 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 figured 61.**

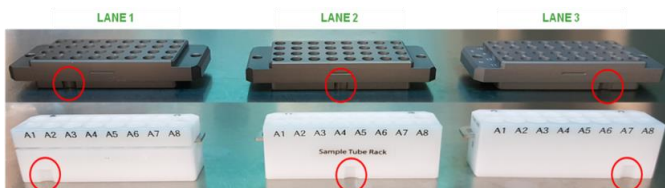


Fig. 59 Checking the position of Elution Tube Rack and Loading Tube Rack

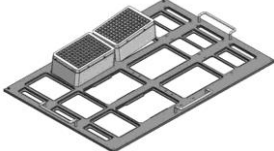
- 6) Fasten the protection cover onto the elution tube rack.



Fig. 60 Installing protection Cover

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

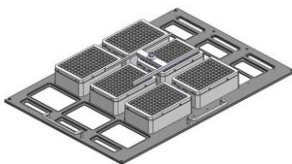
①



Install buffer cartridge ①, ②

to the sample quantity on the set-up tray.

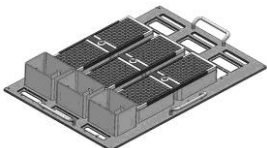
②



Install clamp on top of the buffer cartridge.

Clamps must be installed per lane and hold the clamp.

③



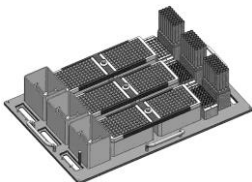
Install the waste tray.

④



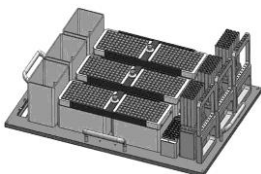
Install elution tube rack that installed PCR Premix Strip and protection cover to the set-up tray.

⑤



Remove the cover of the disposable tip rack and install it on the setup tray.

⑥



Install the 8-hole punch.

- 8) Completed installing components for nucleic acid extraction, prepare control and samples.
- 9) Prepare clinical samples in BSC (Class II,III). 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 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.**

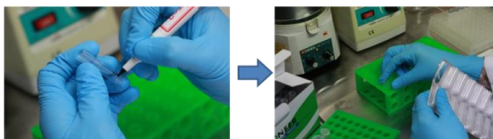


Fig. 61 Necessary components preparing for sample loading




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

 **Before using a Sample Loading Tube, the bottom of the Sample Loading Tube MUST BE checked for Blue color (Dried IPC for DNA)**



**Fig. 62 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  $\mu\text{l}$  of NTC into a tube that is assigned as NTC. (supplied with AccuPower® Diagnostic Kit)
- 13) Additionally add 395  $\mu\text{l}$  SL buffer and 5  $\mu\text{l}$  SPC1~5 into the appropriate SPC wells. (supplied with the AccuPower® Diagnostic Kit)

 **For the pre-date of same lots of Diagnostic kit and Extraction kit, SPC calibration may be skipped. By the Standard, information saves 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 $\mu\text{l}$  in NTC tube.

**LPC:** Load 395 $\mu\text{l}$  SL buffer and 5 $\mu\text{l}$  LPC (blue cap tube, the component of AccuPower® Diagnostic Kit)

**HPC:** Load 395  $\mu\text{l}$  SL buffer and 5  $\mu\text{l}$  HPC (red cap tube, the 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 installs, keep vertical direction during removal and installation of the rack to prevent the pour of loaded solution**

- 15) Load 400  $\mu\text{l}$  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. Confirm the exact position of each Sample Loading Tube, and then set up.
- ⚠ If a clinical sample contaminates gloves or tips, remove the pollutant immediately, then use a new one. 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. Once the tube has been installed, push the fixing device to lock Sample Loading Tube's position.



Fig. 63 The locker to hold the Sample Loading Tube

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

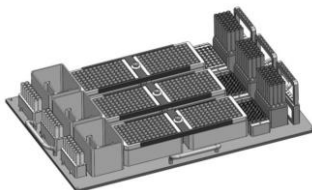


Fig. 64 Install of Sample Tube Rack

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

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

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

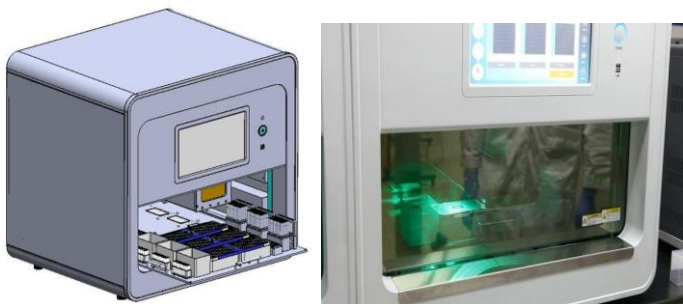


Fig. 65 Install of Setup tray

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



Running time of extraction takes 60-80 minutes according to sample type.



If an error message occurs during the extraction process, please contact the nearest store or the BIONEER International Molecular Diagnosis TS team.



Fig. 66 Start extraction of nucleic acid through 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 nucleic acid extraction, 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, the process after steps. The long delay can lead to the degradation of nucleic acid, which may affect the result value.**

- 2) Refer to '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

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

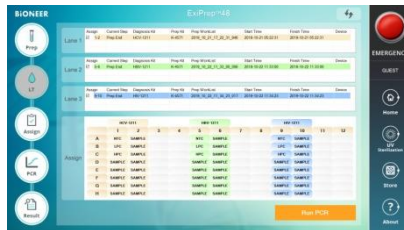


Fig. 67 PCR Random Access

- 4) Click the “Assign” icon. The information list of finished nucleic acid extraction appears on the screen. Select the box for the desired PCR process. Decide the PCR well position according to lane position of *ExiPrep™* 48 Dx.

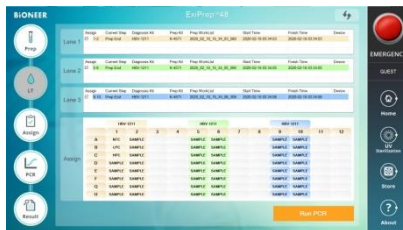


Fig. 68 Select the sample for PCR process

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

- ⚠ **PCR Premix Strip position exactly matches with the assigned position in software.**
- ⚠ **When running PCR under 4 strips, put the balance strip in opposite position to balance of *Exicycler™* 96 thermal block.**

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

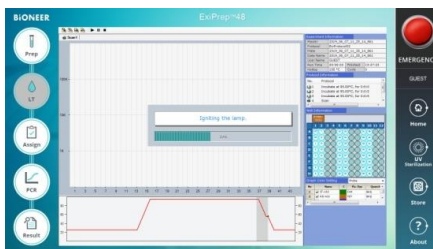


Fig. 70 PCR Running screen

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

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

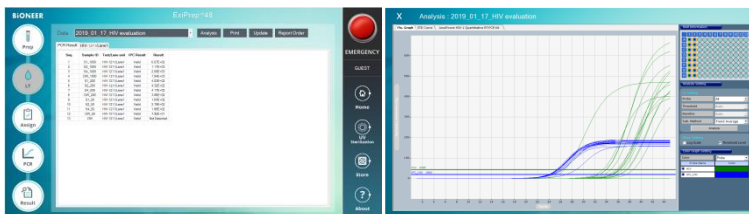


Fig. 71 Data analysis

## 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 Cleanbench-1 for later use.**

**⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedures.**

- 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.

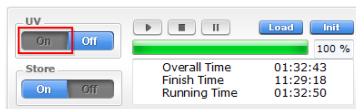


Fig. 72 ExiPrep™ 16 Dx control panel - UV

### 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 Cleanbench-1 for later use.**

**⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedures.**

- 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. 73 ExiPrep™ 48Dx control panel - UV

#### 8.6.4 Exicycler™ 96

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



**Do not peel off an optical sealing film from Diagnostic Kit. discard them according to local safety regulations or internal laboratory procedure**



## 8.7 Data Analysis

### (1) Calibration (HBV SPC (1) – (5))

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

### (2) Control (HBV LPC and HPC)

Every test is provided with controls. The test uses 2 wells of PCs (HPC, LPC) to confirm the validity of each test. The user can check the validity of the test with *ExiStation™* manager software either by the 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 the Ct value of the HBV signal. If the assay is valid, HBV Ct will be 'undetermined' in NTC well and the SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User's Guide section 10. Trouble shooting.

### Specimen results are interpreted as follows:

Titer Result (IU/mL*)	Interpretation
Not Detected	No Ct value (> 45 Ct) of HBV was obtained. Reports are reported as "Not Detected".
<1.50E+01 IU/mL	Calculated IU/mL are below the Limit of Quantification of the assay. Report results as "<1.50E+01".
≥ 1.50E+01 IU/mL and ≤ 1.00E+08 IU/mL	Calculated results greater than or equal to 1.50E+01 IU/mL and less than or equal to 1.00E+08 IU/mL are within the Linear Range of the assay.
>1.00E+08 IU/mL	Calculated IU/mL are above the range of the assay. Report results as "greater than 1.00E+08 IU/mL". If quantitative results are desired, the original specimen should be diluted with HBV-negative human EDTA-plasma or human serum and the test repeated. Multiply the reported result by the dilution factor.

\*IU/mL; HBV DNA concentration in copy/mL X 0.7 IU/copy = HBV DNA in IU/mL

## 8.8 Quality Control

### (1) IPC (Internal Positive Control)

Every test tube contains an IPC to check PCR inhibition by the impurity or the mismanaged thermal cycling to monitor the complete process of analysis. IPC is dried within Sample Loading tube (accessory for nucleic acid extraction, not provide). High concentrations of HBV DNA can lead to a reduced or absent fluorescence signal of the IPC due to PCR competition. The validity of IPC is determined by the Ct value of the IPC signal.

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

The result of IPC determines the validity of the test and the Ct value of the HBV signal determines the HBV concentration (IU/mL) 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® HBV Quantitative PCR Kit was determined by analysis of serial dilutions of the WHO International Standard for HBV DNA for Nucleic Acid Amplification Technology Assays (3<sup>rd</sup> WHO International Standard), in HBV-negative human EDTA plasma Panels of eight dilutions levels plus a negative were tested with three lots of AccuPower® HBV Quantitative PCR Kit and in HBV-negative human serum Panels of eight dilutions levels plus a negative were tested with three lots of AccuPower® HBV Quantitative PCR Kit.

AccuPower® HBV Quantitative PCR Kit detected HBV DNA with a detection rate of 95%, as determined by PROBIT, at a concentration of 6.02 IU/mL in EDTA-plasma and 10.47 IU/mL in serum.

**Table 1. Detection rate of AccuPower® HBV Quantitative PCR Kit at each concentration in EDTA- plasma**

Nominal concentration (Log <sub>10</sub> IU/mL)		Number of replicates tested (N)	Number of positives detected	Positive rate (%)
NTC	0	60	0	0
0.3	-0.51	60	11	18
0.63	-0.20	60	21	35
1.26	0.10	60	36	60
1.86	0.27	60	44	73
2.51	0.40	60	41	68
3.71	0.57	60	54	90
5	0.7	60	58	97
10	1	60	60	100

**Table 2. Detection rate of AccuPower® HBV Quantitative PCR Kit at each concentration in Serum**

Kit Lot	Nominal concentration (Log <sub>10</sub> IU/mL)	Number replicates (N)	of tested	Number of positives detected	Positive rate (%)
NTC	0	72		0	0
0.63	-0.20	72		6	8
1.26	0.10	72		12	17
1.86	0.27	72		36	50
2.51	0.40	71		34	48
3.72	0.57	72		52	72
5.01	0.70	72		56	78
10.00	1	72		66	92
15.14	1.18	72		72	100

**Table 3. Limit of Detection probit analysis in EDTA plasma**

Concept	LoD	95% C.I	
IU/mL	6.02	4.36	7.94
Log <sub>10</sub> IU/mL	0.78	0.65	0.90

**Table 4. Limit of Detection probit analysis in Serum**

Concept	LoD	95% C.I	
IU/mL	10.47	8.30	13.18
Log <sub>10</sub> IU/mL	1.02	0.92	1.12

### 9.1.2 Verification of limit of detection for HBV genotype B - H

The verification of limit of detection of AccuPower® HBV Quantitative PCR Kit detection for genotype B-H was determined by analysis of 5 different dilutions levels of the World-wide HBV DNA Performance Panel (Seracare code: WWHD301, USA) or 1<sup>st</sup> WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques (PEI code 5086/08) in EDTA-plasma (Seracare, Milford, USA) and Serum (Millipore, Darmstadt, Germany) was used.

24-replicate was performed in each dilution and the study results demonstrate that the AccuPower® HBV Quantitative PCR Kit can detect HBV DNA in EDTA plasma and Serum at a concentration as low as 0.78 and 1.00 Log<sub>10</sub> IU/mL, with a positivity rate greater than or equal to 95%.

**Table 5. Detection rate of AccuPower® HBV Quantitative PCR Kit at each concentration in EDTA-plasma.**

Genotype	Concentration (Log <sub>10</sub> IU/ml)	Number replicates tested(N)	of	Number of positive detected	Positive rate (%)
B	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		24	100
	0.70	24		23	96
C	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		24	100
	0.70	24		23	96
D	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		23	96
	0.70	24		23	96
E	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		24	100
	0.70	24		24	100
F	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		23	96
	0.70	24		24	100
G	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		23	96
	0.70	24		20	88
H	1.30	24		24	100
	1.00	24		24	100
	0.88	24		24	100
	0.78	24		23	96
	0.70	24		24	100

Table 6. Detection rate of AccuPower® HBV Quantitative PCR Kit at each concentration in serum

Genotype	Concentration (Log <sub>10</sub> IU/mL)	Number replicates tested(N)	Number of positive detected	Positive rate (%)
B	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
C	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96
D	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
E	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
F	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96
G	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
H	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96

### 9.1.3 Linear range and Limit of Quantitation (LoQ)

Linearity and LoQ of HBV genotype A was performed with a dilution series of the the WHO 3<sup>rd</sup> HBV International Standard panel (NIBSC code: 10/264, UK) for low titer members and HBV DNA plasmid, is test with AccuPower® HBV Quantitative PCR Kit.

The standard panel and HBV DNA plasmid were diluted in HBV negative human EDTA plasma and serum. The concentration of tested sample was from 0.88 Log<sub>10</sub> IU/mL to 8 log<sub>10</sub> IU/mL in EDTA plasma and 1.18 Log<sub>10</sub> IU/mL to 8 log<sub>10</sub> IU/mL in serum.

The evaluation of main LoQ and Linearity was performed with 3 different of Accupower® HBV Quantitative PCR Kit. Test was perform with each concentration 4 replicates and 1 runs per day and on 5 different days, on 3 different ExiStation™ system instruments, resulting in 60 overall data points per dilutions.

Linear range claim for AccuPower® HBV Quantitative PCR Kit was from 1.18 HBV DNA Log<sub>10</sub> IU/mL to at least 8.00 HBV DNA Log<sub>10</sub> IU/mL, with maximum deviation between the observed mean Log<sub>10</sub> titer and the best fitted 1st-order model of less than 0.20 Log<sub>10</sub> for each concentration level tested in this interval. Therefore, the results of this study support the claimed linear range of 1.18 to 8.00 Log<sub>10</sub> IU/mL.

At a concentration of 1.18 Log<sub>10</sub> IU/mL, it was satisfied the total analytical error (TE) value within 1.00 Log<sub>10</sub> IU/mL. Therefore, the claimed LOQ for the AccuPower® HBV Quantitative PCR Kit considering all HBV genotype is 1.18 Log<sub>10</sub> IU/mL.

**Table 7. Linear equation results of HBV genotype in EDTA-plasma**

HBV genotype	Linear equation in genotype linearity study	Maximum difference between HBV genotype A and corresponding HBV genotype (Log <sub>10</sub> IU/mL)	Linear range
A	$y = 1.0749x - 0.2416$	N/A	0.88 Log <sub>10</sub> IU/mL to 8.00 Log <sub>10</sub> IU/mL
B	$y = 1.0675x - 0.2507$	0.07	0.88 Log <sub>10</sub> IU/mL to 4.00 Log <sub>10</sub> IU/mL
C	$y = 1.0425x - 0.165$	0.18	0.88 Log <sub>10</sub> IU/mL to 4.00 Log <sub>10</sub> IU/mL
D	$y = 1.0499x - 0.2189$	0.18	0.88 Log <sub>10</sub> IU/mL to 4.00 Log <sub>10</sub> IU/mL
E	$y = 1.0403x - 0.0075$	-0.19	0.88 Log <sub>10</sub> IU/mL to 4.00 Log <sub>10</sub> IU/mL
F	$y = 1.0268x - 0.0266$	0.17	0.88 Log <sub>10</sub> IU/mL to 4.00 Log <sub>10</sub> IU/mL
G	$y = 1.0542x - 0.1402$	-0.08	0.88 Log <sub>10</sub> IU/mL to 2.00 Log <sub>10</sub> IU/mL
H	$y = 1.0834x - 0.1357$	-0.17	0.88 Log <sub>10</sub> IU/mL to 2.00 Log <sub>10</sub> IU/mL

Table 8. Linear equation results of HBV genotype in serum

HBV genotype	Linear equation in genotype linearity study	Maximum difference between HBV genotype A and corresponding HBV genotype (Log <sub>10</sub> IU/ml)	Linear range
A	$y = 1.058x - 0.3768$	N/A	1.18 Log <sub>10</sub> IU/ml to 8.00 Log <sub>10</sub> IU/ml
B	$y = 1.0733x - 0.3118$	-0.19	1.18 Log <sub>10</sub> IU/ml to 4.00 Log <sub>10</sub> IU/ml
C	$y = 1.0426x - 0.2169$	-0.14	1.18 Log <sub>10</sub> IU/ml to 4.00 Log <sub>10</sub> IU/ml
D	$y = 1.0206x - 0.1703$	-0.16	1.18 Log <sub>10</sub> IU/ml to 4.00 Log <sub>10</sub> IU/ml
E	$y = 1.0615x - 0.2368$	-0.17	1.18 Log <sub>10</sub> IU/ml to 4.00 Log <sub>10</sub> IU/ml
F	$y = 1.0588x - 0.229$	-0.15	1.18 Log <sub>10</sub> IU/ml to 4.00 Log <sub>10</sub> IU/ml
G	$y = 1.036x - 0.2114$	-0.14	1.18 Log <sub>10</sub> IU/ml to 2.00 Log <sub>10</sub> IU/ml
H	$y = 1.065x - 0.2915$	-0.14	1.18 Log <sub>10</sub> IU/ml to 2.00 Log <sub>10</sub> IU/ml

Table 9. LOQ results of HBV genotypes in EDTA-plasma

HBV Genotype	Nominal concentration (Log <sub>10</sub> IU/ml)	N	Average Measured Concentration (Log <sub>10</sub> IU/ml)	Bias  (Log <sub>10</sub> IU/ml)	SD (Log <sub>10</sub> IU/ml)	TE =  Bias  + 2 x SD (Log <sub>10</sub> IU/ml)	SQRT[2]x2 x SD (Log <sub>10</sub> IU/ml)
A	1.18	60	0.97	0.21	0.20	0.61	0.56
B	1.18	30	0.99	0.19	0.16	0.51	0.46
C	1.18	30	0.92	0.26	0.28	0.81	0.79
D	1.18	30	1.02	0.16	0.18	0.52	0.51
E	1.18	30	1.09	0.09	0.17	0.43	0.49
F	1.18	30	1.26	0.08	0.21	0.50	0.59
G	1.18	30	1.04	0.14	0.15	0.44	0.43
H	1.18	30	1.00	0.18	0.22	0.61	0.61

**Table 10. LOQ results of HBV genotypes in serum**

HBV Genotype	Nominal concentration (Log <sub>10</sub> IU/ml)	N	Average Measured Concentration (Log <sub>10</sub> IU/ml)	Bias  (Log <sub>10</sub> IU/ml)	SD (Log <sub>10</sub> IU/ml)	TE =  Bias  + 2 x SD (Log <sub>10</sub> IU/ml)	SQRT[2]x2 x SD (Log <sub>10</sub> IU/ml)
A	1.18	60	0.73	0.45	0.25	0.94	0.69
B	1.18	30	0.94	0.24	0.29	0.82	0.83
C	1.18	30	1.09	0.09	0.22	0.53	0.62
D	1.18	30	0.98	0.20	0.28	0.76	0.79
E	1.18	30	1.08	0.27	0.31	0.89	0.88
F	1.18	30	0.97	0.20	0.31	0.83	0.88
G	1.18	30	1.04	0.13	0.15	0.44	0.43
H	1.18	30	1.07	0.11	0.28	0.66	0.78

#### 9.1.4 Precision

Precision claim for AccuPower® HBV Quantitative PCR Kit was determined by analysis of international standard panel(3<sup>rd</sup> WHO International Standard) and HBV plasmid (BIONEER corp.). 3 dilution levels were tested in 80 replicates for each level across three lots of AccuPower® HBV Quantitative PCR Kit using *ExiStation™* system for 20 days in EDTA-plasma and in serum(for repeatability).

The between-Lot experiment was conducted in 3 lots, 2 replicates per samples, and 2 runs per day during 5 days. The between-operator and between-instrument experiment was conducted in 1 lots, 2 replicates per samples, and 2 runs per days, 2 operator(or instrument) on during 5 days. The between-site experiment was conducted in 1 lot, 2 replicates per samples, and 2 runs per day on during 5 days.(for reproducibility).

**Table 11.The summary result of repeatability in EDTA-plasma**

Nominal Concentration (Log <sub>10</sub> IU/ml)	Assigned Concentration (Log <sub>10</sub> IU/ml)	No. of Valid tests	Within-Run(S <sub>r</sub> )	Between-Run(S <sub>rr</sub> )	Between-Day(S <sub>dd</sub> )	Total precision(S <sub>r</sub> )
6.00	6.44	80	0.09	0.04	0.06	0.12
3.33	3.46	80	0.10	0.03	0.02	0.11
1.26	1.23	80	0.16	0.10	0.10	0.21



Table 12.The summary result of repeatability in serum

Nominal Concentration (Log <sub>10</sub> IU/mL)	Assigned Concentration (Log <sub>10</sub> IU/mL)	No. of Valid tests	Within-Run(S <sub>r</sub> )	Between-Run(S <sub>rr</sub> )	Between-Day(S <sub>dd</sub> )	Total precision(S <sub>T</sub> )
6.00	5.65	80	0.15	0.13	0.07	0.21
3.33	3.19	80	0.10	0.04	0.03	0.11
1.65	1.33	80	0.18	0.10	0.13	0.24

Table 13.The result of reproducibility in EDTA-plasma

Plasma			Standard Deviation			
Nominal Concentration (Log <sub>10</sub> IU/mL)	Assigned Concentration (Log <sub>10</sub> IU/mL)	No. of Valid Tests	Between-Lot	Between-Site	Between-Operator	Between-Instrument
6.00	6.43	200	0.11	0.13	0.10	0.08
3.33	3.47	200	0.09	0.12	0.10	0.10
1.26	1.26	200	0.20	0.21	0.22	0.22

Table 14.The result of reproducibility in serum

Serum			Standard Deviation			
Nominal Concentration (Log <sub>10</sub> IU/mL)	Assigned Concentration (Log <sub>10</sub> IU/mL)	No. of Valid Tests	Between-Lot	Between-Site	Between-Operator	Between-Instrument
6.00	5.66	200	0.21	0.18	0.17	0.19
3.33	3.21	200	0.10	0.13	0.09	0.09
1.65	1.34	200	0.22	0.26	0.21	0.26

### 9.1.5 Interfering substances

Interfering effects by twenty-two (22) exogenous substances (included anti-viral substance) and by seven (7) endogenous substances was tested for interfering of AccuPower® HBV Quantitative PCR kit.

Potentially interfering endogenous and exogenous substances were spiked into EDTA plasma and into serum in the absence or presence of 2 (3.33, 1.26 Log<sub>10</sub> IU/mL in Plasma and 3.33, 1.65 Log<sub>10</sub> IU/mL in Serum) concentration of HBV and were compared to control EDTA plasma and serum samples containing no spiked interfering substance. Each concentration level for each interfering substance was tested in three (3) and eight(8) replicates.

All tested interfering substance concentrations was shown no influence on the performance of the AccuPower® HBV Quantitative PCR Kit at the HBV concentrations.

**Table 15. Interference- Exogenous Interfering Substances**

No.	Potential substance interfering	Concentration
1	Raltegravir	25.8 mg/L
2	Entecavir	164ng/ml
3	Lamivudine	44.8ug/ml
4	Telbivudine	74ug/ml
5	Rifampicin	312mg/L
6	Trimethoprim	2760umol/ml
7	Isoniazid	5.84mmol/ml
8	Rifabutin	9.2mg/ml
9	Adefovir dipivoxil	368ng/L
10	Sulfamethoxazole	31.6mmol/L
11	Pyrazinamide	576ug/ml
12	Nevirapine	40ug/ml
13	Ritonavir	224ug/L
14	Ribavirin	54.96ug/L
15	Abacavir	60ug/ml
16	Tenofovir	6ug/ml
17	Zidovudine	45.8ug/ml
18	Efavirenz	81.4ug/ml
19	Saquinavir	104.16ug/ml
20	Nelfinavir	40ug/ml
21	Valganciclovir	113ug/ml
22	Amprenavir	153.2ug/ml

**Table 16. Interference- Endogenous Interfering Substances**

No.	Potential interfering substance	Concentration
1	EDTA	540mg/dL
2	Citrate	0.372M/ml
3	Heparin	600U/ml
4	Hemoglobin	40mg/ml
5	Cholesterol	200mg/ml
6	Albumin	10g/dL
7	Bilirubin	0.25mg/ml

### 9.1.6 Cross reactivity

The following viruses and Bacteria were tested for cross-reactivity of AccuPower® HBV Quantitative PCR Kit. Samples were prepared by diluting organisms either in HBV negative EDTA-plasma and in HBV negative serum or in HBV panel spiked EDTA-plasma and in HBV spiked serum and were tested in eight(8) or three(3) replicates. negative HBV EDTA plasma and serum samples for negative were shown negative and HBV positive specimens spiked in cross-reactivity organisms were shown to detect within  $\pm 0.44 \text{ Log}_{10} \text{ IU/mL}$  in EDTA-plasma and  $\pm 0.65 \text{ Log}_{10} \text{ IU/mL}$  in serum

**Table 17. List of potential cross reactivity organism**

Viruses		Bacteria
Hepatitis A virus	Zika Virus	Mycobacterium gordonae
HIV-1	Human herpesvirus 6B	Chlamydia trachomatis
Hepatitis C virus	Human herpesvirus 8	Neisseria gonorrhoeae
Epstein-Barr Virus	HIV-2	Staphylococcus aureus
Cytomegalovirus	Adenovirus type 5	
Human papillomavirus 16	Dengue virus types 1	
Human papillomavirus 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)	
Varicella-Zoster Virus	Influenza Virus A(H3N2)	
West Nile Virus		

### 9.1.7 Whole system failure rate

The Whole System Failure rate was tested with one-hundred twenty(120) replicates using AccuPower® HBV Quantitative PCR Kit. Positive results were obtained 100% detection of one-hundred twenty(120) replicates; overall, a system success rate was shown 100% in the AccuPower® HBV Quantitative PCR Kit in plasma and serum respectively.

Matrix	Concentration ( $\text{Log}_{10} \text{ IU/mL}$ )	Test number	Detection rate(%)
Plasma	1.26	120	100%
Serum	1.51	120	100%

### 9.1.8 Cross contamination

Cross-contamination test was performed using HBV diagnostic kit according to the CTS guideline. High positive and negative were tested at 7.00 Log<sub>10</sub> IU/mL concentration and Negative HBV free matrix, respectively.

The AccuPower® HBV Quantitative PCR Kit showed no evidence of cross-contamination when tested with eighty (80) HBV positive samples (7.00 Log<sub>10</sub> IU/mL) and negative samples, which were loaded in an alternating order. Ten (10) extraction and ten (10) PCR runs were performed in total.

Run	Number of samples (detected/ tested)		Sample information(Log <sub>10</sub> IU/mL)		Pass/fail
	Positive	Negative	7.00	Negative	
Run1	8/8	0/8	6.79	Not detected	Pass
Run2	8/8	0/8	6.85	Not detected	Pass
Run3	8/8	0/8	6.70	Not detected	Pass
Run4	8/8	0/8	6.79	Not detected	Pass
Run5	8/8	0/8	6.80	Not detected	Pass
Run6	8/8	0/8	6.68	Not detected	Pass
Run7	8/8	0/8	6.70	Not detected	Pass
Run8	8/8	0/8	6.78	Not detected	Pass
Run9	8/8	0/8	6.59	Not detected	Pass
Run10	8/8	0/8	6.74	Not detected	Pass
Average			6.74	-	-
SD			0.12	-	-

### 9.1.9 Matrix Equivalency

A total of thirty(30) paired samples(serum and EDTA plasma samples) that was collected from HBV positive patients and HBV DNA positive were used for matrix equivalency with AccuPower® HBV Quantitative PCR Kit. The slope of deming regression was shown a 1.0034 (95% confidence interval [0.970 to 1.036]) with an intercept of 0.2458 (95% confidence interval [0.131 to 0.360]).

Log<sub>10</sub> IU/mL titer difference (mean log titer serum – mean log titer EDTA plasma) value of thirty(30) paired samples was shown within 0.5 log<sub>10</sub> IU/mL. as mean difference value was shown -0.26 Log<sub>10</sub> IU/mL(95% CI: 0.19 and 0.31), there were not significantly different between serum and EDTA-plasma.

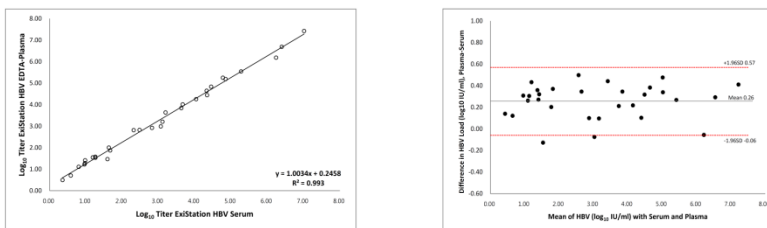


Fig. 48 PCR Running screen

Key Statistics		Deming Regression Statistics	
		Y=Slope * X + Intercept	
correlation coefficient (R)	0.9965	Slope	1.0034(0.970 to 1.036)
coefficient of determination (R <sup>2</sup> )	0.993	Intercept	0.2458 (0.131 to 0.360)
Bias Mean	0.26(0.19 to 0.31)	Std.Err of Estimate	0.162
Bias Std Dev.	0.16	Points (Plotted/Total)	30/30
Mean difference±2SD	-0.06 to 0.57	Outliers	0

## 9.2 Diagnostic Performance Characteristics

### 9.2.1 Diagnostic Sensitivity & Specificity

Total two- hundred- forty-four (244) clinical samples, which were confirmed with other HBV quantitative assay, were used for diagnostic sensitivity& specificity test.

The percent agreement between the test device under evaluation and the comparison device was calculated as follows:

		Reference system		
		Positive	Negative	Total
ExiStation™ system	Positive	133	0	133
	Negative	0	111	111
	Total	133	111	244

Diagnostic Sensitivity (Percent positive agreement) = **100 % (95% C.I 97.19 – 100)**

Diagnostic Specificity (Percent negative agreement) = **100 % (95% C.I 96.65 – 100)**

### 9.2.2 Correlation

Total one-hundred thirty-three (133) HBV positive EDTA plasma clinical samples were compared with CE-IVD approved HBV NAT assay.

The scatter plot for two assays is shown in correlation results graph, with the linear equation  $y = -0.529 + 1.048 X$  and correlation coefficient  $R^2 = 0.935$ , which indicated a significant correlation between the two assays.

The Bland Altman plot analysis was used to assess the difference between the positive results obtained with the two assays (Bland Altman graph). The results showed that 95.49% of positive data were within the 95% acceptable range (-0.44 to 1.16), the average difference value was 0.36.

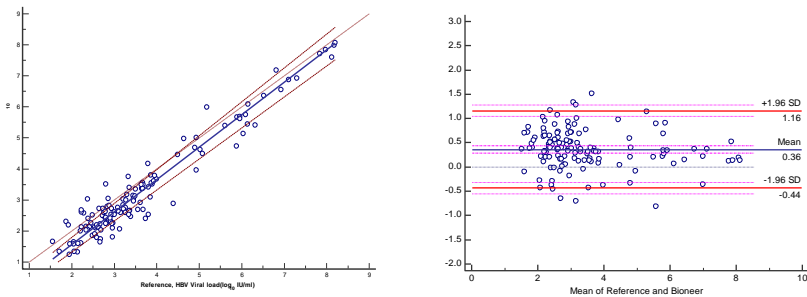


Fig. 49 Correlation with CE-IVD approved assay

### 9.2.2 Verification of precision

Precision was validated by manufacturer. the results of manufacturer`s precision claim was verified in clinical site. This study was analyzed two dilution of HBV international standard panel that was tested with one lot of AccuPower® HBV Quantitative PCR Kit according to CLSI EP15-A. Two replicates of each dilution per day were tested at each dilution for 3 days. The user`s verification results of precision was shown that user`s verification results was lower than manufacturer`s precision claim.

The  $S_{within}$  or  $S_{Total}$  precision of the AccuPower® HBV Quantitative PCR Kit assay was verified to be consistent with the manufacturer`s claim.

**Table 18. summary results of User`s precision verification.**

Concentration	Analytical performance		Verification performance		Verification performance	
	Precision value		Precision value		verification value	
	$\sigma_{within}$	$\sigma_{Total}$	$S_{within}$	$S_{Total}$	$S_{within}$	$S_{Total}$
1.26 log <sub>10</sub> IU/mL	0.16	0.21	0.16	0.14	0.25	0.33
3.33 log <sub>10</sub> IU/mL	0.10	0.11	0.10	0.09	0.16	0.16

10. TROUBLESHOOTING

Comments and suggestions

Internal Positive Control (IPC) invalid results	
<p>If the TAMRA (IPC) Fluorescence signal was not detected in all wells (including controls)</p>	<ul style="list-style-type: none"> <li>Extraction and/or PCR configuration error                             <ul style="list-style-type: none"> <li>☞ Make sure that the correct extraction/PCR protocol was programmed and performed in accordance with the Kits. Repeat the assay, if necessary.</li> <li>See <b>User's Guide 8. PROTOCOL</b></li> </ul> </li> <li>Incorrect extraction or PCR kit use                             <ul style="list-style-type: none"> <li>☞ Make sure that you use proper kits for the intended tests.</li> </ul> </li> <li>The kit may have spoiled, due to bad storage or expiration.                             <ul style="list-style-type: none"> <li>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.</li> <li>See <b>User's Guide 5. STORAGE AND SHELF LIFE</b></li> </ul> </li> <li>Invalid results.                             <ul style="list-style-type: none"> <li>☞ It must be tested with the new reagent</li> </ul> </li> </ul>
<p>If the TAMRA (IPC) Fluorescence signal was not detected in particular wells.</p>	<ul style="list-style-type: none"> <li>Inhibition of PCR                             <ul style="list-style-type: none"> <li>☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition.</li> <li>☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type.</li> </ul> </li> <li>Low elution volume due to insoluble material of samples                             <ul style="list-style-type: none"> <li>☞ 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.</li> </ul> </li> </ul>



### 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 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 AND SHELF LIFE**
  - 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 AND SHELF LIFE**
- 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.

**invalid result of True positive sample or suspected sample**

If true positive sample in HBV qualification test was not quantified a HBV viral load or

If suspected sample was not quantified a HBV viral load.

- There may have been a nucleic acid error
  - ☞ In case of suspected sample, It must be tested with adjustable HBV screening assay.
  - ☞ In case of true positive sample, It must be re-tested with the new reagent.

## 11. REFERENCES

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## 12. SYMBOLS



Catalog number



Temperature limitation



In vitro diagnostic medical device



Contains sufficient for test



Manufacturer



Caution, consult accompanying documents



Batch code



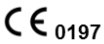
Expiration date



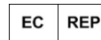
Do not reuse



Consult instructions for use



Conformite Europeenne Mark



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Warning for hazardous and irritation



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