REF 201300 NeuMoDx™ HBV Quant Test Strip

℞ only

CAUTION: For US Export Only

IVD For *in vitro* diagnostic use with the NeuMoDx 288 and NeuMoDx 96 Molecular Systems

For insert updates, go to: www.qiagen.com/neumodx-ifu
For detailed instructions, refer to the NeuMoDx 288 Molecular System Operator's Manual; P/N 40600108
For detailed instructions, refer to the NeuMoDx 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx HBV Quant Assay is an automated, in vitro nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human plasma and serum specimens for HBV genotypes A through H of HBV-infected individuals. The NeuMoDx HBV Quant Assay implemented on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx System(s)) incorporates automated DNA extraction to isolate target nucleic acid from the specimen and real-time polymerase chain reaction (qPCR) to target the highly conserved sequences in the Hepatitis B viral genome.

The NeuMoDx HBV Quant Assay is intended for use as an aid in the management of patients with HBV infections. The results from the NeuMoDx HBV Quant Assay must be interpreted within the context of all relevant clinical and laboratory findings. The NeuMoDx HBV Quant Assay is not intended for use as a screening test for blood or blood products or as a diagnostic tool to diagnose the clinical status of HBV infection.

SUMMARY AND EXPLANATION

Human whole blood collected in sterile blood collection tubes containing either ethylenediaminetetraacetic acid (EDTA) or acid citrate-dextrose (ACD) as anticoagulation agents or in plasma preparation tubes (PPT) may be used for the preparation of plasma, while serum should be collected in serum collection tubes or separation tubes (SST). To prepare for testing, plasma or serum in a secondary specimen tube or fractionated blood in a primary specimen tube compatible with the NeuMoDx System is loaded onto the NeuMoDx System using a designated specimen tube carrier. For each specimen, an aliquot of plasma or serum sample is mixed with NeuMoDx Lysis Buffer 1 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the products of amplification (sections of the HBV genome target in the highly conserved region encoding *X protein* and *preC* protein). The NeuMoDx HBV Quant Assay includes a DNA Sample Process Control (SPC1) to help monitor for the presence of potential inhibitory substances as well as NeuMoDx System or reagent failures that may be encountered during the extraction and amplification processes.

Hepatitis B virus (HBV) is the causative agent of hepatitis B liver infection and is a global health problem. Hepatitis B can cause either acute hepatitis or can progress to a chronic condition resulting in cirrhosis or liver cancer. The risk for a chronic condition developing is primarily age related; if the virus is transmitted at birth, there is a > 90% chance that a chronic condition will develop while an adult becoming infected has a 2-6% chance of developing a chronic condition.[1] HBV is transmitted either through blood to blood contact with an infected person, through sexual transmission, sharing needles with an infected person in IV drug use, or vertical transmission from mother to baby during childbirth. In the United States, approximately 850,000 people are living with HBV infection, with the majority of new infections resulting from sexual transmission or injection-drug use.[2] In Africa and the Western Pacific, as many as 5% of the population are known to be infected. Worldwide in 2015, HBV infection caused 885,000 deaths, mostly from cirrhosis or hepatocellular carcinoma.[3] A vaccine exists that is 95% effective at preventing HBV infection thereby resulting in fewer diagnosed cases each year.[4]

The current standard of care for treating HBV infection is antiviral therapy, which requires constant monitoring to ensure treatment is progressing as desired. Monitoring of therapy using the NeuMoDx HBV Quant Assay can provide physicians with information necessary to aid in managing patients with HBV infections.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx HBV Quant Assay combines automated DNA extraction, amplification, and detection by real-time PCR. Whole blood specimens are collected in EDTA, ACD, or PPT tubes for the preparation of plasma and/or into SST tubes for the preparation of serum. The primary (fractionated) blood specimen or a plasma/serum aliquot in a compatible secondary specimen tube is barcoded and placed on the NeuMoDx System. The NeuMoDx System automatically aspirates an aliquot of the plasma/serum to mix with NeuMoDx Lysis Buffer 1 and the agents contained in the NeuMoDx Extraction Plate to begin processing. The NeuMoDx System automates and integrates DNA extraction and concentration, reagent preparation, and nucleic acid amplification/detection of the target sequences using real-time PCR. The included Sample Process Control (SPC1) helps monitor for the presence of inhibitory substances and for system, process, or reagent failures. No operator intervention is necessary once the specimen is loaded onto the NeuMoDx System.

The NeuMoDx System uses a combination of heat, lytic enzyme, and extraction reagents to automatically perform lysis, DNA extraction, and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles, with bound nucleic acid, are loaded into the NeuMoDx Cartridge where the unbound elements are washed away with NeuMoDx Wash Reagent. The bound DNA is then eluted using NeuMoDx Release Reagent. The NeuMoDx System uses the eluted DNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the HBV and SPC1 targets. This enables simultaneous amplification and detection of both target and control DNA sequences. Upon reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences (if present) occur in the PCR chamber. The NeuMoDx Cartridge is designed to contain the amplicon following PCR, virtually eliminating the risk of post-amplification contamination.

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) with fluorogenic oligonucleotide probe molecules specific to the amplicons of their respective targets. TaqMan probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe is intact, the fluorophore and the quencher are in proximity, allowing the quencher molecule to suppress the fluorescence emitted by the fluorophore via Förster Resonance Energy Transfer (FRET).

TaqMan probes are designed such that they anneal within an DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore and breaks its proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing detection of the fluorophore. The resulting fluorescent signal detected in the NeuMoDx System quantitative PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target present.

A TaqMan probe labeled with a fluorophore (Excitation: 490 nm & Emission: 521 nm) at the 5' end and a dark quencher at the 3' end, is used to detect HBV DNA. For detection of the SPC1, the TaqMan probe is labeled with an alternate fluorescent dye (Excitation: 535 nm & Emission: 556 nm) at the 5' end and a dark quencher at the 3' end. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software analyzes the data and reports a final result (POSITIVE/NEGATIVE/INDETERMINATE/UNRESOLVED/NO RESULT). If a result is positive and the calculated concentration is within the limits of quantitation, the NeuMoDx System software also provides a quantitative value associated with the sample.

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
201300	**NeuMoDx HBV Quant Test Strip** *Dried PCR reagents containing HBV and SPC1 specific TaqMan probe and primers*	6	16	96

Materials Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
800100 or 800102	**NeuMoDx HBV Calibrators** *Single use sets of HBV High and Low Calibrators to establish validity of calibration curve*
900101 or 900102	**NeuMoDx HBV External Controls** *Single use sets of Positive and Negative Controls*
400400	**NeuMoDx Lysis Buffer 1**
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100100	**NeuMoDx Cartridge**
235903	**Hamilton® CO-RE/CO-RE II Tips (300 µL) with Filters**
235905	**Hamilton CO-RE/CO-RE II Tips (1000 µL) with Filters**

Instrumentation Required

NeuMoDx 288 Molecular System [REF 500100] or **NeuMoDx 96 Molecular System** [REF 500200]

WARNINGS AND PRECAUTIONS

- The NeuMoDx HBV Quant Test Strip is for *in vitro* diagnostic use with NeuMoDx Systems only.
- Do not use the reagents or consumables after the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use consumables or reagents if the protective pouch is open or broken upon arrival.
- A valid test calibration (generated by processing high and low calibrators from the NeuMoDx HBV Calibrators) must be available before test results can be generated for clinical samples.
- NeuMoDx HBV External Controls must be processed every 24 hours throughout testing with the NeuMoDx HBV Quant Assay.
- Minimum specimen volume is dependent on the tube size, specimen carrier, and specimen volume processing as defined below. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.

- The use of specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results.
- Avoid microbial and deoxyribonuclease (DNase) contamination of all reagents and consumables at all times. The use of sterile DNase-free disposable transfer pipettes is recommended if using secondary tubes. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from the Biohazard Waste Container (NeuMoDx 288 Molecular System) or Biohazard Waste Bin (NeuMoDx 96 Molecular System) under any circumstances. The NeuMoDx Cartridge is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx HBV Quant Test Strip, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of the NeuMoDx Cartridge, the foil seal surface of the NeuMoDx HBV Quant Test Strip and NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 1; handling of the consumables and reagents should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are provided for each reagent (as applicable) at www.qiagen.com/neumodx-ifu.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[5] and in CLSI Document M29-A4.[6]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.
- Do not reuse.

PRODUCT STORAGE, HANDLING AND STABILITY

- NeuMoDx HBV Quant Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 4 to 28 °C.
- Do not use consumables and reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- Do not reload any test product that has previously been loaded onto another NeuMoDx System.
- Once loaded, the NeuMoDx HBV Quant Test Strip may remain onboard the NeuMoDx System for 62 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the System.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

1. Handle all specimens, calibrators, and controls as if they are capable of transmitting infectious agents.
2. Do not freeze whole blood or any specimens stored in primary tubes.
3. To prepare plasma specimens, whole blood should be collected in sterile tubes using EDTA or ACD as the anticoagulants. Follow the specimen collection tube manufacturer instructions for preparation and storage.
4. To prepare serum specimens, whole blood should be collected in SST tubes. Follow the specimen collection tube manufacturer instructions for preparation and storage.
5. Specimens may be tested in primary collection tubes or secondary specimen tubes. Recommended for primary tube testing:
    a. Plasma specimens: BD Vacutainer® Plus Plastic K_2EDTA Tube (BD #368589) or BD Vacutainer PPT™ Plasma Preparation Tube (BD #362799).
    b. Serum specimens: BD Vacutainer Plus Plastic Serum Tube (BD #367820) or BD Vacutainer SST™ Tube (BD #367988).
6. Prepared specimens may be stored on the NeuMoDx System for up to 8 hours for plasma and 24 hours for serum prior to processing. If additional storage time is required, it is recommended that the specimens be either refrigerated or frozen as secondary aliquots.
7. Prepared specimens should be stored between 2–8 °C for no longer than 7 days prior to testing and a maximum of 8 hours for plasma and 24 hours for serum at room temperature.
8. Prepared specimens may be stored at ≤ -20 °C for up to 4 weeks (serum) or 6 months (plasma) before processing; frozen specimens should not undergo more than 2 freeze/thaw cycles for plasma and 4 freeze/thaw cycles for serum prior to use.
    a. If samples are frozen, allow the samples to completely thaw at room temperature (15–30 °C); vortex to generate a uniformly distributed sample.
    b. Once frozen samples are thawed, testing should occur within 24 hours.
    c. Freezing of plasma/serum in primary collection tubes is not recommended.
9. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.
10. Label specimens clearly and indicate specimens are for HBV testing.

11. Proceed to *Test Preparation* section.

The overall process for implementation of the NeuMoDx HBV Quant Assay is summarized below in *Figure 1*.

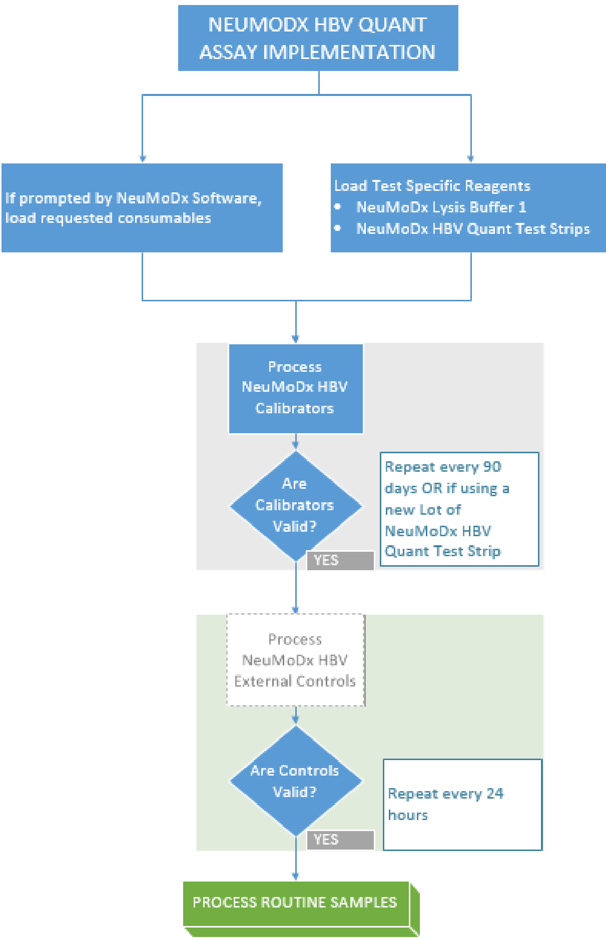

Figure 1: NeuMoDx HBV Quant Assay Implementation Workflow

INSTRUCTIONS FOR USE

Test Preparation

The NeuMoDx HBV Quant Assay can be run directly from primary blood collection tubes or from specimen aliquots in secondary tubes. Processing can be run using one of two specimen volumes processing workflows—550 µL specimen volume workflow or 200 µL specimen processing workflow. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System. The primary blood collection tube may be labeled and placed directly into a 32-tube Specimen Tube Carrier, following centrifugation as directed by the manufacturer. Alternatively, an aliquot of the plasma/serum may be transferred to a secondary tube for processing on the NeuMoDx System.

2. If testing the specimen in the primary collection tube, place the barcoded tube into a Specimen Tube Carrier and ensure the cap is removed prior to loading onto the NeuMoDx System. Minimum volumes ***above*** gel/buffy layer are defined below and will be met if specimens are collected and processed according to tube manufacturer instructions. Performance is not guaranteed for specimens that are collected improperly.

Tube Type	Minimum Required Specimen Volume	
	550 μL Workflow	200 μL Workflow
SST – 3.5 mL	1550 μL	1200 μL
PPT/SST – 5.0 mL	1800 μL	1450 μL
PPT/SST – 8.5 mL	2500 μL	2200 μL
K_2EDTA/Serum – 4.0 mL	1050 μL	700 μL
K_2EDTA/Serum – 6.0 mL	1250 μL	900 μL
K_2EDTA/Serum – 10.0 mL	1600 μL	1250 μL

3. If using a secondary tube, transfer an aliquot of the plasma/serum to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:

Specimen Tube Carrier	Tube Size	Minimum Required Specimen Volume	
		550 μL Workflow	200 μL Workflow
32-Tube Specimen Tube Carrier	11–14 mm diameter by 60–120 mm height	700 μL	400 μL
24-Tube Specimen Tube Carrier	14.5–18 mm diameter by 60–120 mm height	1100 μL	800 μL
Low Volume Specimen Tube Carrier	1.5 mL conical bottom microcentrifuge tube	650 μL	300 μL

NeuMoDx Systems Operation

For detailed instructions, refer to the NeuMoDx 288 and 96 Molecular Systems Operator's Manuals (P/N 40600108 & 40600317)

1. Load the test order onto the NeuMoDx System according to the desired specimen processing volume workflow and specimen tube type:
    - 550 μL specimen volume is tested by defining the specimen type as "**Plasma**" or **Serum**"
    - 200 μL specimen volume is tested by defining the specimen type as "**Plasma2**" or "**Serum2**"
    - If not defined in the test order, the **Plasma** specimen type in a **Secondary Tube** will be used as default
2. Populate one or more NeuMoDx System Test Strip carrier(s) with NeuMoDx HBV Quant Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
3. If prompted by the NeuMoDx System software, add the necessary required consumables to the NeuMoDx System consumable carriers and use the touchscreen to load carrier(s) into the NeuMoDx System.
4. If prompted by the NeuMoDx System software, replace NeuMoDx Wash Reagent, NeuMoDx Release Reagent, empty the Priming Waste, Biohazard Waste Container (NeuMoDx 288 Molecular System only), Tip Waste Bin (NeuMoDx 96 Molecular System only), or Biohazard Waste Bin (NeuMoDx 96 Molecular System only), as appropriate.
5. If prompted by the NeuMoDx System software, process NeuMoDx HBV Calibrators and/or NeuMoDx HBV External Controls. Further information regarding calibrators and controls can be found in the *Results Processing* section.
6. Load the specimen/calibrator/control tube(s) into a Specimen Tube Carrier and ensure caps are removed from all tubes.
7. Place the Specimen Tube Carrier(s) on the autoloader shelf and use the touchscreen to load the carrier(s) into the NeuMoDx System. This will initiate processing of the loaded specimens for the tests identified, given a valid test order is present in the system.

LIMITATIONS

1. The NeuMoDx HBV Quant Test Strip can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx HBV Quant Test Strip has been established for plasma specimens prepared with EDTA/ACD as anti-coagulants or serum specimens prepared in serum separator tubes. Use of the NeuMoDx HBV Quant Test Strip with other sources has not been assessed and performance characteristics are unknown for other specimen types.
3. The performance of the NeuMoDx HBV Quant Test Strip has been established for primary tube testing using BD Vacutainer Plus Plastic K_2EDTA Tubes, BD Vacutainer PPT Plasma Preparation Tube, BD Vacutainer Plus Plastic Serum Tube, and BD Vacutainer SST Tube.
4. A small increase in the limit of detection and lower limit of quantitation of the NeuMoDx HBV Quant Assay has been observed when using 200 μL specimen volume workflow.

5. The NeuMoDx HBV Quant Assay is used for quantitative monitoring purposes only. It is not meant to be used for qualitative detection.
6. The NeuMoDx HBV Quant Assay must not be used with samples from heparinized humans.
7. Because detection of HBV is dependent on the number of target DNA particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
8. NeuMoDx HBV Calibrators and NeuMoDx HBV External Controls must be processed as recommended in the package inserts when prompted by NeuMoDx System software before processing routine clinical samples.
9. Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube confusion. In addition, false negative results could occur because the number of viral particles in the sample is below the limit of detection of the NeuMoDx HBV Quant Assay.
10. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
11. If both HBV target and the SPC1 target do not amplify, an invalid result (Indeterminate, No Result, or Unresolved) will be reported and the test should be repeated.
12. If the NeuMoDx HBV Quant Assay result is Positive, but the quantitation value is beyond the limits of quantitation, the NeuMoDx System will report whether detected HBV was *below* Lower Limit of Quantitation (LLoQ) or *above* Upper Limit of Quantitation (ULoQ).
13. In the event the detected HBV was *below* LLoQ, the assay may be repeated (if desired) with another aliquot of the specimen.
14. In the event the detected HBV is above ULoQ, the NeuMoDx HBV Quant Assay may be repeated with a diluted aliquot of the original specimen. A 1:1000 dilution in HBV negative plasma or Basematrix 53 Diluent (Basematrix) (SeraCare, Milford, MA) is recommended. The concentration of the original specimen can be calculated as follows:

    original specimen concentration = log_{10}(dilution factor) + reported concentration of the diluted sample

15. The occasional presence of PCR inhibitors in plasma may result in a system quantitation error. If this occurs, it is recommended that the test be repeated with the same specimen diluted in Basematrix at 1:10 or 1:100.
16. A positive result does not necessarily indicate the presence of viable organisms. However, a positive result is presumptive for the presence of hepatitis B virus DNA.
17. Deletions or mutations in the conserved regions targeted by the NeuMoDx HBV Quant Assay may affect detection or could lead to an erroneous result using the NeuMoDx HBV Quant Test Strip.
18. Results from NeuMoDx HBV Quant Assay should be used as an adjunct to clinical observations and other information available to the physician; the assay is not intended to diagnose infection.
19. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.

RESULTS PROCESSING

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx HBV Quant Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx HBV Quant Assay Definition File (HBV ADF). A result may be reported as Negative, Positive with a reported HBV concentration, Positive above ULoQ, Positive below LLoQ, Indeterminate (IND), Unresolved (UNR), or No Result (NR), based on the amplification status of the target and sample process control. Results are reported based on the ADF decision algorithm, summarized below in *Table 1*.

Table 1: Summary of the HBV Quant Assay Decision Algorithm

RESULT	HBV Target	Sample Process Control (SPC1)	Result Interpretation
Positive with Reported Concentration	Amplified 0.9 ≤ [HBV] ≤ 9.0 $\log_{10}$ IU/mL (550 µL Workflow) 1.4 ≤ [HBV] ≤ 9.0 $\log_{10}$ IU/mL (200 µL Workflow)	Amplified or Not Amplified	HBV DNA detected within quantitative range
Positive, above ULoQ	Amplified [HBV] > 9.0 $\log_{10}$ IU/mL	Amplified or Not Amplified	HBV DNA detected above quantitative range
Positive, below LLoQ	Amplified [HBV] < 0.9 $\log_{10}$ IU/mL (550 µL Workflow) [HBV] < 1.4 $\log_{10}$ IU/mL (200 µL Workflow)	Amplified or Not Amplified	HBV DNA detected below quantitative range
Negative	Not Amplified	Amplified	HBV DNA not detected
Indeterminate	Not Amplified, System Error Detected, Sample Processing Completed		All target results were invalid; retest sample†
No Result*	Not Amplified, System Error Detected, Sample Processing Aborted		Sample processing was aborted; retest sample†
Unresolved	Not Amplified, No System Error Detected		All target results were invalid; retest sample†

*No Result flag is only reported on NeuMoDx System software versions 1.8 and higher
†The NeuMoDx System is equipped with automatic Rerun/Repeat capability that the end user can choose to use to ensure that an IND/UNR/NR result is automatically reprocessed to minimize delays in result reporting.

Test Calculation

1. For samples within the Quantitation range of the NeuMoDx HBV Quant Assay, the concentration of HBV DNA in the samples is calculated using the stored standard curve in conjunction with the calibration coefficient and specimen volume.
    a. A calibration coefficient is calculated based on the results of the NeuMoDx HBV Calibrators processed to establish validity of the standard curve for a given lot of the NeuMoDx HBV Quant Test Strip on a specific NeuMoDx System.
    b. The calibration coefficient is incorporated into the final determination of the concentration of HBV DNA.
    c. The NeuMoDx Software accounts for the specimen input volume when determining the concentration of HBV DNA per mL of specimen.
2. NeuMoDx HBV Quant Assay results are reported in $\log_{10}$ IU/mL.
3. The resulting quantitation of the unknown samples is traceable to the WHO 4th HBV International Standard.

Test Calibration

A valid calibration based on the Standard Curve is required to quantitate HBV DNA in the specimens. To generate valid results, a test calibration must be completed using the external calibrators provided by NeuMoDx Molecular, Inc.

Calibrators

1. A set of NeuMoDx HBV calibrators need to be processed with each new lot of NeuMoDx HBV Quant Test Strips, if a new HBV Quant Assay Definition File is uploaded to the NeuMoDx System, if the current set of calibrators are past the validity period (currently set at 90 days), or if the NeuMoDx System software is modified.
2. The NeuMoDx System software will notify the user when calibrators need to be processed. A new lot of test strips cannot be used for testing until the calibrators have been processed successfully.
3. Calibration validity is established as follows:
    a) A set of two calibrators – one (1) high and one (1) low – need to be processed to establish validity.
    b) At least two (2) out of the three (3) replicates must give results within predefined parameters. The low calibrator nominal target is 3.7 $\log_{10}$ IU/mL and the high calibrator nominal target is 5.7 $\log_{10}$ IU/mL.
    c) A calibration coefficient is calculated to account for expected variation between test strip lots. This calibration coefficient is utilized in determination of final HBV concentration.
4. If one or both the calibrators fail the validity check, repeat processing of the failed calibrator(s) using a new vial. In the event one calibrator fails validity, it is possible to only repeat the failed calibrator as system does not require the user to run both calibrators again.

5. If the calibrator(s) fail the validity check consecutively, contact NeuMoDx Molecular, Inc.

Quality Control

Local regulations typically specify that the laboratory is responsible for control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, approved test system.

External Controls

1. Positive and negative external controls need to be processed every 24 hours throughout testing with the NeuMoDx HBV Quant Assay. If a set of valid external control results does not exist, the NeuMoDx System software will prompt the user for controls to be processed before sample results can be reported.
2. Validity of external controls will be assessed by the NeuMoDx System based on the expected result. The positive control should provide an HBV Positive result and the negative control should provide an HBV Negative result.
3. Discrepant result handling for external controls should be performed as follows:
   a) A Positive test result reported for a negative control sample indicates a specimen contamination problem.
   b) A Negative test result reported for a positive control sample may indicate there is a reagent or instrument related problem.
   c) In either of the above instances, or in the event of an indeterminate (IND) result or No Result (NR), repeat the NeuMoDx HBV External Controls with fresh vials of the control(s) failing the validity test.
   d) If positive NeuMoDx HBV external control continues to report a Negative result, contact NeuMoDx technical service.
   e) If negative NeuMoDx HBV external control continues to report a Positive result, attempt to eliminate all sources of potential contamination, including replacing all reagents before contacting NeuMoDx technical service.

Sample Process (Internal) Controls

An exogenous Sample Process Control (SPC1) is incorporated in the NeuMoDx Extraction Plate and undergoes the entire process of nucleic acid extraction and real-time PCR amplification with each sample. Primers and probe specific to SPC1 are also included in each NeuMoDx HBV Quant Test Strip, enabling detection of SPC1 with the target HBV DNA (if present) via multiplex PCR. Detection of SPC1 amplification allows the NeuMoDx System software to monitor the efficacy of the DNA extraction and PCR amplification processes.

Invalid Results

If a NeuMoDx HBV Quant Assay performed on the NeuMoDx System fails to produce a valid result following completion of sample processing, it will be reported as Indeterminate (IND), No Result (NR), or Unresolved (UNR) based on the type of error that occurred.

An IND result will be reported if a NeuMoDx System error is detected during sample processing. In the event an IND result is reported, a retest is recommended.

An UNR result will be reported if no valid amplification of HBV DNA or SPC1 is detected in the absence of system errors, indicating possible reagent failure or the presence of inhibitors. If an UNR result is reported, a retest is recommended as a first step. If a retest fails, a specimen dilution may be used to mitigate the effects of any sample inhibition.

If a NeuMoDx HBV Quant Assay performed on the NeuMoDx System fails to produce valid result and sample processing is aborted prior to completion, it will be reported as a No Result (NR). In the event a NR is reported, a retest is recommended.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity – Limit of Detection using the WHO Standard

The Analytical Sensitivity of the NeuMoDx HBV Quant Assay was characterized by testing negative specimens and a dilution series of the WHO 4th International Standard in screened negative human plasma and serum to determine the Limit of Detection (LoD) on the NeuMoDx Systems. The LoD was defined as the lowest target level detected at a rate of 95% as determined by Probit-style analysis. The studies were performed over 3 days across multiple NeuMoDx Systems with multiple lots of NeuMoDx reagents. An additional study was executed to confirm the LoD of the NeuMoDx HBV Quant Assay when using the 200 µL specimen volume workflow. Detection rates from both studies are shown in *Table 2.*

Table 2: Positive Detection Rates for LoD Determination of the NeuMoDx HBV Quant Assay

	Target Concentration [IU/mL]	Target Concentration [$\log_{10}$ IU/mL]	PLASMA			SERUM		
			Number of Valid Tests	Number of Positives	Detection Rate	Number of Valid Tests	Number of Positives	Detection Rate
550 µL	20	1.30	108	108	100%	107	107	100%
	10	1	108	107	99%	108	104	96%
	5	0.70	108	98	91%	108	95	88%
	2.5	0.40	108	97	90%	108	72	67%

	1.25	0.10	108	73	68%	108	44	42%
	NEG	N/A	108	0	0%	107	0	0%
200 µL	25	1.40	43	43	100%	44	44	100%

The LoD of the NeuMoDx HBV Quant Assay for HBV Genotype A (4th WHO International Standard) in plasma was determined to be 5.2 IU/mL (95% CI 4.1 – 7.6 IU/mL) [(0.72 $\log_{10}$ IU/mL) (95% CI 0.61 – 0.88 $\log_{10}$ IU/mL)] using the 550 µL specimen volume workflow (*Figure 2*). The LoD of the NeuMoDx HBV Quant Assay for serum specimens was determined to be 8.0 IU/mL (95% CI 6.5 – 10.8 IU/mL) [(0.9 $\log_{10}$ IU/mL) (95% CI 0.8 – 1.0 $\log_{10}$ IU/mL)] using the 550 µL specimen volume workflow (*Figure 2*).

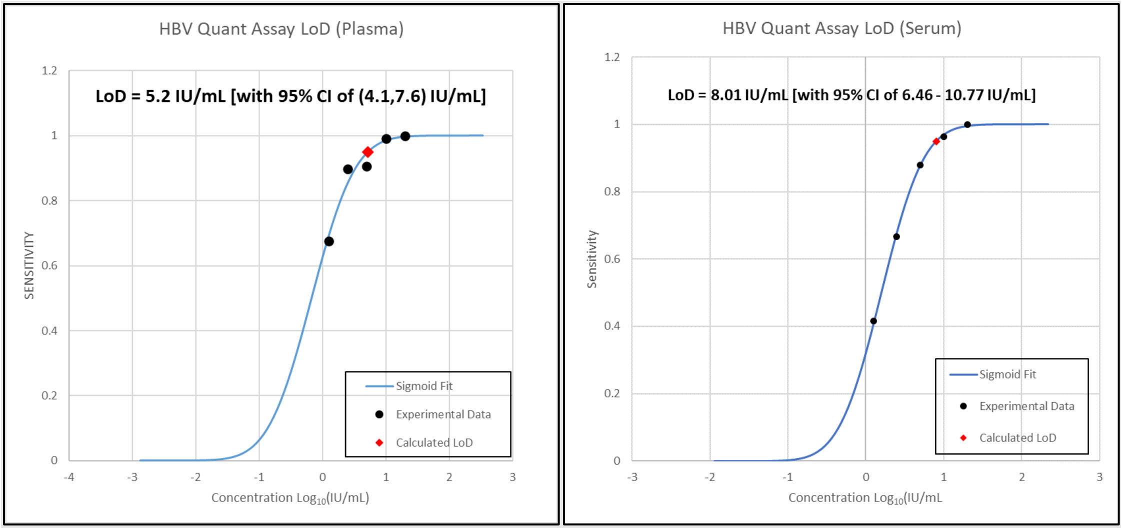

Figure 2: Probit style analysis used to determine the LoD of the NeuMoDx HBV Quant Assay, Plasma (left) and Serum (right)

Analytical Sensitivity – Quantitation Limit – Lower Limit of Quantitation (LLoQ) using the WHO Standard

The Lower Limit of Quantitation (LLoQ) is defined as the lowest target level at which > 95% detection is achieved AND the TAE ≤ 1.0. To determine the LLoQ, the total analytical error (TAE) was calculated for each of the HBV target levels that were shown to report > 95% detection as part of LoD calculation. TAE is defined as follows:

TAE = bias + 2*SD [Westgard Statistic]

The bias is the absolute value of the difference between the average of calculated concentration and the expected concentration. SD refers to the standard deviation of the quantitated value of the sample.

Compiled results for the 5 levels of HBV specimens used in the LLoQ study using the 4th WHO International Standard are shown in *Table 3*. The LLoQ for the 4th WHO International Standard in plasma using the NeuMoDx HBV Quant Assay (550 µL specimen volume workflow) was determined to be 5.5 IU/mL (0.74 $\log_{10}$ IU/mL). A separate study was performed to confirm the LLoQ when using the 200 µL specimen volume workflow and these results demonstrated an LLoQ of 25 IU/mL , which is also shown in *Table 3.*

The LLoQ of the NeuMoDx HBV Quant Assay for serum specimens was determined to be 6.0 IU/mL using the 550 µL specimen volume workflow, and 25 IU/mL for the low volume (200 µL) specimen volume workflow as shown in *Table 3*.

Table 3: NeuMoDx HBV Quant Assay LLoQ, with Bias and TAE

	Target Conc. [IU/mL]	Target Conc. [$\log_{10}$ IU/mL]	Plasma					Serum				
			Average Conc. [$\log_{10}$ IU/mL]	Detection (%)	SD	Bias	TAE	Average Conc. [$\log_{10}$ IU/mL]	Detection (%)	SD	Bias	TAE
550 µL	20	1.30	1.29	100	0.23	0.16	0.63	1.43	100	0.20	0.13	0.52
	10	1.00	1.07	99	0.25	0.20	0.71	1.21	96	0.24	0.21	0.69
	5	0.70	0.89	91	0.35	0.34	1.04	1.00	88	0.40	0.30	1.09
	2.5	0.40	0.75	90	0.44	0.51	1.39	0.96	67	0.44	0.56	1.46
	1.25	0.10	0.73	68	0.41	0.68	1.50	0.95	42	0.38	0.85	1.61
200 µL	25	1.40	1.61	100	0.35	0.21	0.91	1.81	100	0.18	0.41	0.78

Analytical Sensitivity - LoD and LLoQ across HBV Genotypes

The LoD was initially established for Genotype A (4th WHO International Standard) and then additional testing was performed around the established LoD using each of the other 7 genotypes. Thirty six (36) replicates at levels corresponding to 2X, 1X and 0.5X of the 95% CI upper limit of LoD (~7 IU/mL) were tested using the NeuMoDx HBV Quant Assay using plasma with the 550 µL specimen volume workflow. The positive percentage rate for each genotype at each of these tested levels was tabulated and used to calculate the LoD using a Probit-style analysis.

The Total Analytical Error at these levels tested was also calculated. The lowest level with 95% positive detection and calculated TAE of ≤ 1.0 was again considered to be the LLoQ for the genotype. Across genotypes, the Limit of Detection of the NeuMoDx HBV Quant Assay for plasma specimens using the 550 µL specimen volume workflow was demonstrated to be 6.2 IU/mL (0.79 $\log_{10}$ IU/mL) and the LLoQ was demonstrated to be 7.6 IU/mL (0.88 $\log_{10}$ IU/mL), as shown in *Table 4*.

Table 4. HBV Genotypes Tested in Plasma using 550 µL Specimen Volume Workflow

GENOTYPE	LoD [IU/mL]	LLoQ [IU/mL]
Genotype A	5.2	5.2
Genotype B	6.2	6.2
Genotype C	3.5	6.2
Genotype D	5.2	5.7
Genotype E	3.5	3.5
Genotype F	5.1	6.2
Genotype G	3.5	3.5
Genotype H	5.2	7.6

Based on the outcome of these studies, NeuMoDx claims an **LoD and LLoQ of 25 IU/mL (1.4 $\log_{10}$ IU/mL)** for the NeuMoDx HBV Quant Assay in ***plasma and serum*** using the **200 µL specimen volume workflow**.

NeuMoDx claims an **LoD and LLoQ of 8.0 IU/mL (0.9 $\log_{10}$ IU/mL)** for the NeuMoDx HBV Quant Assay in ***plasma and serum*** using the **550 µL specimen volume workflow**.

Analytical Sensitivity – Linearity and Determination of Upper Limit of Quantitation (ULoQ)

Linearity and the Upper Limit of Quantitation (ULoQ) of the NeuMoDx HBV Quant Assay were established in plasma by preparing a dilution series using high positive HBV Clinical Sample (Access Biologicals, Vista, CA) with established traceability to the 4th WHO International Standard. An 11-member panel was prepared in pooled HBV negative plasma to create a testing panel that would span a concentration range of 9.02-1.02 $\log_{10}$ IU/mL. The Testing Panel was processed with 6 replicates at each level across 2 NeuMoDx Systems and 3 lots of critical reagents. The NeuMoDx HBV Quant Assay demonstrated the ability to quantify HBV across the 8 $\log_{10}$ linear range (including critical medical decision points) with a deviation of ±0.22 $\log_{10}$ IU/mL. No significant benefit was gained using 2nd and 3rd order regression fits. The ULoQ was determined using the data from this study to be 9.02 $\log_{10}$ IU/mL [*Table 5* and *Figure 3*].

Table 5: Linearity of the NeuMoDx HBV Quant Assay (evaluated with Genotype A)

Target Conc. (IU/mL)	Target Conc. (log_{10} IU/mL)	Mean Conc. (log_{10} IU/mL)	Standard Deviation	Bias	Predicted Linear Fit	Deviation from Non-Linear Fit
1.05E+09	9.02	8.99	0.08	0.06	9.02	-0.04
1.05E+08	8.02	8.05	0.07	0.05	8.02	0.03
1.05E+07	7.02	7.05	0.07	0.06	7.02	0.04
1.05E+06*	6.02	6.05	0.05	0.05	6.02	0.03
1.05E+05	5.02	5.04	0.05	0.04	5.02	0.00
2.82E+04*	4.45	4.43	0.07	0.05	4.45	-0.01
1.05E+04	4.02	3.99	0.09	0.05	4.02	-0.02
2.82E+03*	3.45	3.41	0.07	0.06	3.45	-0.03
1.05E+03	3.02	3.00	0.04	0.04	3.02	-0.03
1.05E+02	2.02	1.99	0.11	0.09	2.02	-0.01
1.05E+01	1.02	1.09	0.29	0.23	1.02	0.06

**Near Medical Decision Points*

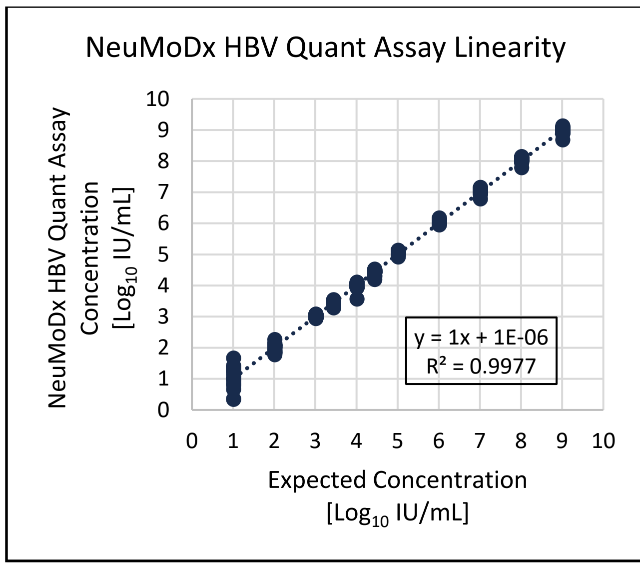

Figure 3: Linear range of the NeuMoDx HBV Quant Assay in Plasma

A subsequent study was performed to demonstrate matrix equivalency and the analysis compared the NeuMoDx HBV quantitative results for samples prepared in plasma and serum using two different regression fit models, including MS Excel regression tool and Passing-Bablok. Results showed a strong correlation represented by slope and intercept values very close to 1.00 and 0.00 respectively, and an R^2 value of 0.99 (MS Excel Regression Tool) or a p-value of 0.270 (Passing-Bablok). The HBV Quant assay concentrations reported by the NeuMoDx System for the plasma matrix compared to corresponding serum samples are presented in *Figure 4.*

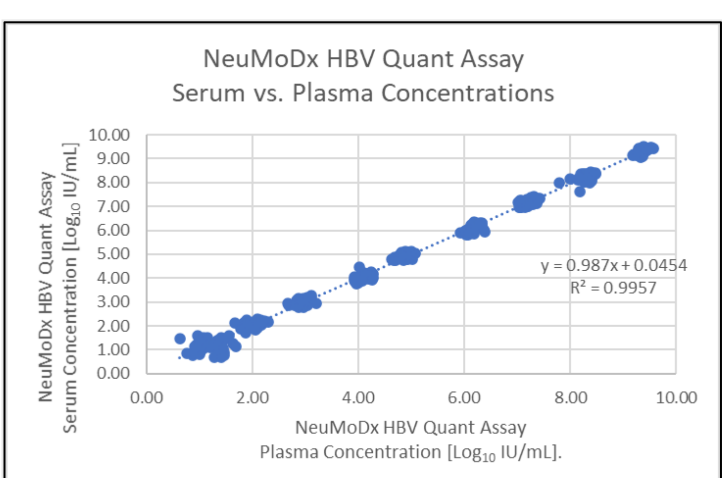

Figure 4: Linear range of the NeuMoDx HBV Quant Assay between Matrices

Linearity and ULoQ were then confirmed for the 200 µL specimen volume workflow over a range of 9.31–1.71 log_{10} IU/mL. Equivalency comparisons were performed between the concentrations reported by the NeuMoDx Software for the 200 µL and 550 µL workflows. Deming and Passing-Bablok regression analysis showed excellent correlation and a slope close to 1 and minimum intercepts (bias) of the reported concentrations for both plasma and serum samples across the linear range. A Bland and Altman comparison of the reported concentration for the 200 µL specimen volume workflow to the mean reported concentration for both 200 µL and 550 µL specimen volume workflows showed minimal bias, attributing accuracy to the algorithm used to generate results from the 200 µL workflow. Additionally, a simple linear regression comparing the expected concentration to the reported concentration for the 200 µL workflow had a slope close to 1, demonstrating excellent correlation [*Figure 5]*. Taken together, these comparisons demonstrate accurate quantitation of HBV across the linear range of the NeuMoDx HBV Quant Assay using the 200 µL specimen volume workflow.

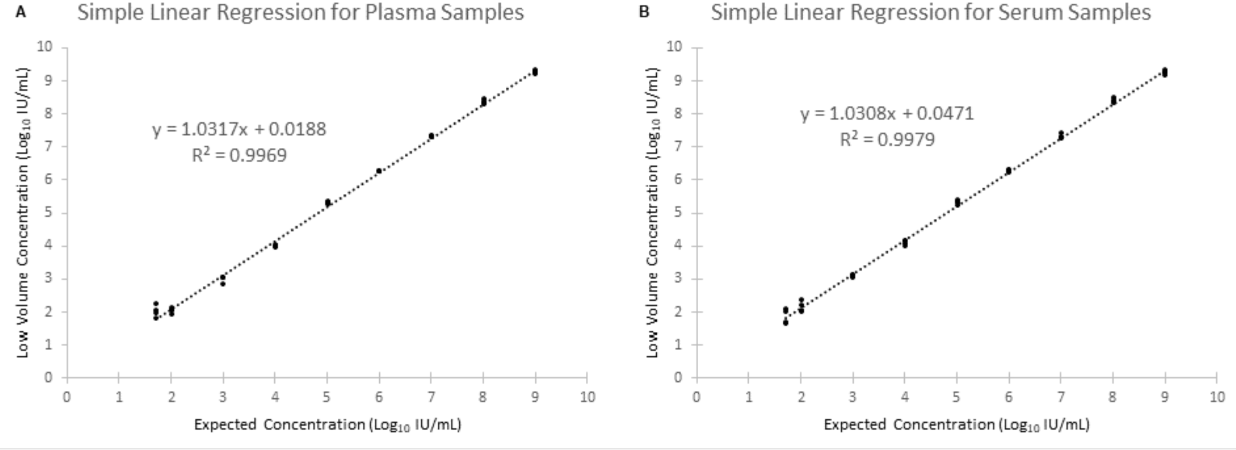

Figure 5: Linear Relationship between Expected and NeuMoDx Reported Concentrations for the 200 µL Workflow in a) Plasma and b) Serum

Linearity Across Genotypes

The linearity of the NeuMoDx HBV Quant Assay in plasma specimen for the HBV genotypes was characterized by testing at least four (4) different concentrations of each genotype of HBV prepared in pooled HBV-negative plasma. The tested levels of HBV target used in this study were dependent on the concentration of the source specimen, and therefore differed across genotypes. The study was performed with each genotype using 6 replicates at each level. Linearity across the HBV genotypes is presented in *Table 6* and *Figure 6.*

Table 6: Linearity of the NeuMoDx HBV Quant Assay Across Genotypes

Genotype	Linearity Equation y = NeuMoDx HBV Quant Assay Quantitation x = Expected Quantitation	R^2
A	y = 1x + 1E-06	0.9977
B	y = 1.0129x - 0.0964	0.9975
C	y = 1.0250x - 0.0898	0.998
D	y = 1.0379x - 0.0896	0.9975
E	y = 1.0182x - 0.096	0.9956
F	y = 0.9736x + 0.276	0.9906
G	y = 1.0547x - 0.0835	0.9813

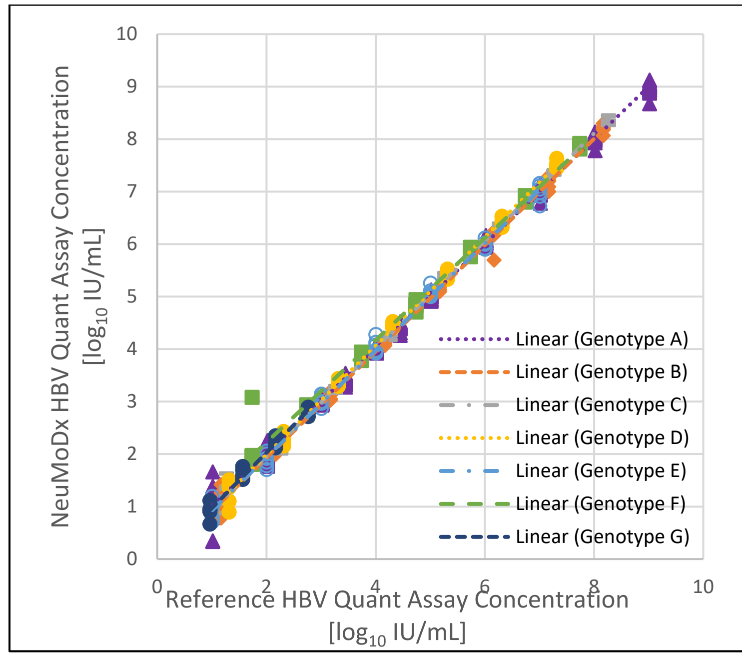

Figure 6: Linearity of the NeuMoDx HBV Quant Assay across Genotypes

Analytical Specificity and Cross-reactivity

Analytical specificity was demonstrated by screening 32 organisms commonly found in blood/plasma specimens as well as species phylogenetically similar to HBV for cross-reactivity**.** Organisms were prepared in pools of between 4 and 6 organisms and tested at a high concentration. The organisms tested are shown in *Table 7*. No cross-reactivity was observed with any of the organisms tested confirming 100% analytical specificity of the NeuMoDx HBV Quant Assay.

Table 7: Pathogens Used to Demonstrate Analytical Specificity – Cross Reactivity

Adenovirus 2	Dengue V1	Hepatitis A	HPV 16	Ilheus (ILHV)	Yellow Fever
Adenovirus 5	Dengue V2	Hepatitis C	HPV 18	Influenza A	Zika Virus
Banzi Virus	Dengue V3	Human Herpesvirus 6a	HSV1	Parvo B19	
BK Virus	Dengue V4	Human Herpesvirus 8	HSV 2	Rubella	
Cytomegalovirus	Epstein Barr Virus	HIV 1	HTLV 1	St. Louis Encephalitis	
VZV	Vaccinia Virus	HIV 2	HTLV 2	West Nile Virus	

Interfering Substances - Commensal Organisms

The NeuMoDx HBV Quant Assay was evaluated for interference in the presence of non-target organisms using the same organism pools as the ones prepared for testing analytical specificity. The organisms were either tested individually or pooled in groups of 4-6 organisms in screened HBV negative plasma and spiked with HBV controls at a concentration of 3.7 $\log_{10}$ IU/mL. No significant interference was observed in the presence of these commensal organisms as indicated by the minimal deviation of quantitation from control specimens which contained no interfering agent [*Table 8*].

Table 8: Interference Testing – Commensal Organisms

Non-Target Organisms	Average conc. ($\log_{10}$ IU/mL)	Bias ($\log_{10}$ IU/mL)
Group 1 [BK Virus, Cytomegalovirus, Epstein Barr Virus, Human Herpesvirus 6a, Human herpesvirus 8]	3.51	0.10
Group 2 [Adenovirus 2, Adenovirus 5, Dengue V2, Dengue V3, Dengue V4]	3.38	0.22
Group 3 [Parvo B19, HTLV 1, HTLV 2, Ilheus (ILHV), Yellow Fever, Zika Virus]	3.62	0.06
Group 4 [HPV 16, HPV 18, HSV 1, HSV 2, Dengue V1]	3.57	0.04
Group 5 [St. Louis Enceph, VZV, Vaccinia Virus, West Nile Virus]	3.57	0.03
HAV	3.58	0.05
Banzi virus	3.41	0.19
HIV-1	3.47	0.15
HIV-2	3.56	0.05
Rubella	3.16	0.44
Influenza A	3.60	0.03
HCV	3.58	0.04

Interfering Substances - Endogenous and Exogenous Substances

The performance of the NeuMoDx HBV Quant Test Strip was assessed in the presence of typical exogenous and endogenous interfering substances encountered in HBV clinical plasma specimens. These included abnormally high levels of blood components as well as common antiviral medications, which were classified *Table 9*. Each of the endogenous and exogenous substances listed below in *Table 10* was added to screened HBV-negative human plasma spiked with 3.7 $\log_{10}$ IU/mL HBV and data observed for interference. In addition, common disease state plasma associated with Hepatitis B infection were also tested for potential interference.

Table 9: Interference Testing - Exogenous Agents (Drug Classifications)

Pool	Drug	Classification
1	Zidovudine (ZDV)	Reverse Transcriptase Inhibitor
	Saquinavir	HIV Protease Inhibitor
	Ritonavir	HIV Protease Inhibitor
	Clarithromycin	Antibiotic
	Interferon alfa-2a	Immune Modulator
	Interferon alfa-2b	Immune Modulator
2	Abacavir sulfate	Reverse Transcriptase Inhibitor
	Amprenavir	Protease Inhibitor
	Ribavirin	Immune Modulator
	Entecavir	HBV Antiviral
	Fluoxetine	SSRI Antidepressant
	Valacyclovir hydrochloride	Antiviral
3	Tenofovir disoproxil	HBV/HIV Antiviral
	Lamivudine	HBV/HIV Antiviral
	Ganciclovir	CMV Antiviral
	Valganciclovir	CMV Antiviral
	Nevirapine	Reverse Transcriptase Inhibitor
4	Efavirenz	Reverse Transcriptase Inhibitor
	Lopinavir	Protease Inhibitor
	Enfuvirtide	HIV Fusion Inhibitor
	Ciprofloxacin	Antibiotic
	Paroxetine	SSRI Antidepressant
5	Adefovir (dipivoxil)	Antiviral
	Azithromycin	Antibiotic
	Indinavir sulfate	HIV Protease Inhibitor
	Sertraline	SSRI Antidepressant

Table 10: Interference Testing - Exogenous and Endogenous Agents

Endogenous	Average Conc. (log10 IU/mL)	Bias (log10 IU/mL)
Hemoglobin	3.50	0.20
Triglycerides	3.51	0.09
Bilirubin	3.56	0.13
Albumin	3.51	0.17
Exogenous (Drugs)	**Average Conc. (log10 IU/mL)**	**Bias (log10 IU/mL)**
Pool 1: Zidovudine (ZDV), Saquinavir, Ritonavir, Clarithromycin, Interferon alfa-2a, Interferon alfa-2b	3.58	0.08
Pool 2: Abacavir sulfate, Amprenavir, Ribavirin, Entecavir, Fluoxetine, Valacyclovir Hydrochloride	3.56	0.04
Pool 3: Tenofovir disoproxil, Lamivudine, Ganciclovir, Valganciclovir, Nevirapine	3.59	0.06
Pool 4: Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin, Paroxetine,	3.60	0.07
Pool 5: Adefovir (dipivoxil), Azithromycin, Indinavir sulfate, Sertraline	3.56	0.19
Disease State	**Average Conc. (log10 IU/mL)**	**Bias (log10 IU/mL)**
Antinuclear Antibody (ANA)	3.61	0.10
Systemic Lupus Erythematosus (SLE)	3.63	0.10
Rheumatoid Arthritis (RA)	3.57	0.09
HCV Antibodies	3.58	0.07
HBV Antibodies	3.64	0.11
Alcoholic cirrhosis	3.68	0.15
Rheumatoid Factor (RF)	3.63	0.10
Non-Alcoholic Steatohepatitis (NASH)	3.49	0.06

Within Lab Precision

Precision of the NeuMoDx HBV Quant Test Strip was determined by testing an 8-member panel of HBV specimens spanning genotypes A and C using three NeuMoDx Systems across 12 days. The within-run, within-day and within-System precisions were characterized, and the overall standard deviation was determined to be ≤ 0.22 $\log_{10}$ IU/mL. Precision between operators was not characterized as the operator plays no significant role in the processing of samples using the NeuMoDx System. Within Lab Precision results are presented in *Table 11*.

Table 11: Within Lab Precision Study Results

PANEL MEMBER	TARGET CONC. [Log10 IU/mL]	MEAN CONC. [Log10 IU/mL]	N	Bias	Within Run SD	Within Day SD	Within System SD	Overall SD
Genotype A	7.75	7.89	36	0.14	0.06	0.07	0.07	0.07
	5.75	5.83	36	0.11	0.07	0.10	0.10	0.10
	3.75	3.70	36	0.11	0.11	0.13	0.15	0.15
	1.75	1.54	36	0.23	0.16	0.22	0.22	0.22
Genotype C	6.27	6.23	36	0.10	0.08	0.09	0.10	0.10
	4.27	4.18	36	0.08	0.08	0.10	0.10	0.10
	3.27	3.14	36	0.09	0.08	0.12	0.12	0.12
	2.27	2.08	36	0.12	0.12	0.15	0.16	0.16

Lot to Lot Reproducibility

Lot to lot Reproducibility of the NeuMoDx HBV Quant Test Strip was determined using three different lots of key reagents – NeuMoDx Lysis Buffer 1, NeuMoDx Extraction Plate and the NeuMoDx HBV Quant Test Strip. An 8-member panel of HBV-genotypes A and C was used to assess performance. Testing was performed using the three lots of reagents on three NeuMoDx Systems across 6 days. The variation within and across lots was analyzed. Maximum overall bias was 0.12 $\log_{10}$ IU/mL and maximum overall SD was 0.24 $\log_{10}$ IU/mL. No significant difference was found in performance across lots as quantitation of all panel members was within tolerance specification. Lot to Lot Reproducibility results are presented below in *Table 12*.

Table 12: Lot to Lot Reproducibility Study Results

PANEL MEMBER	TARGET CONC. [$\log_{10}$ IU/mL]	MEAN CONC. [$\log_{10}$ IU/mL]	N	Bias	Within LOT SD	Across LOTS SD	Overall SD
Genotype A	8.02	7.99	36	0.03	0.15	0.09	0.17
	6.02	5.96	36	0.06	0.17	0.06	0.18
	4.02	3.90	36	0.12	0.14	0.09	0.17
	2.02	1.92	36	0.10	0.21	0.12	0.24
Genotype C	6.27	6.32	36	0.05	0.06	0.08	0.10
	4.27	4.31	36	0.04	0.22	0.09	0.24
	3.27	3.30	36	0.03	0.20	0.07	0.21
	2.27	2.32	36	0.05	0.13	0.13	0.18

Effectiveness of Control

The efficacy of the SPC1 included in the NeuMoDx HBV Quant Assay to report any process step failures or inhibition affecting performance of the NeuMoDx HBV Quant Assay was assessed using two common HBV genotypes (A and C). The conditions tested are representative of critical process step failures that could potentially occur during sample processing and *may not be detected* by the NeuMoDx System performance monitoring sensors. Effectiveness of SPC1 was evaluated by simulating such failure conditions. Process inefficiencies that had an adverse effect on HBV detection/quantitation were mirrored by performance of SPC1 target (Presence of Inhibitor and Lack of Wash step). For conditions under which SPC1 amplification was not affected, the HBV Target was also shown to be amplified within a reported quantitation of 0.2 $\log_{10}$ IU/mL of the control samples.

Table 13: Effectiveness of the Sample Process Control

Process Step Failure Tested	Sample Process Control Amplification Status	HBV Target Amplification Status	Assay Result
Presence of Inhibitor	Not Amplified	Not Amplified	Unresolved
No Wash Delivered	Not Amplified	Not Amplified	Unresolved
No Wash Blowout	Amplified	Amplified	Positive with Quantitation within 0.2 $\log_{10}$ IU/mL of Control

Cross-contamination

The cross-contamination rate the NeuMoDx HBV Quant Assay was determined by testing three sets of HBV specimens featuring alternating high positive and negative specimens. In total, this involved testing 144 replicates of a normal, HBV-negative human EDTA-plasma specimen and 144 replicates of a high-titer HBV specimen at 8.0 $\log_{10}$ IU/mL. All 144 replicates of the negative specimen were negative, which demonstrates no cross-contamination occurred during sample processing on the NeuMoDx System.

Specimen Matrix Equivalence

Testing was performed to demonstrate equivalent results with plasma specimens collected in both ethylenediaminetetraacetic acid (EDTA) and acid citrate dextrose (ACD) collection tubes. Additionally, testing to demonstrate equivalency between fresh and frozen specimens was performed. Forty individual donor specimens, sourced from BioIVT, were collected into both EDTA and ACD collection tubes. These fresh samples were spiked with four levels of HBV genotype A or C and tested for equivalency. The samples were then frozen for a minimum of 24 hours, thawed and re-tested. Excellent equivalency was demonstrated between fresh and frozen specimens and EDTA and ACD specimens by regression analysis.

Table 14: Regression Analysis of Specimen Equivalency Results

Parameter[Acceptance Criteria]	Fresh vs Frozen	ACD vs K2EDTA
Slope [0.9-1.1]	1.002	0.996
Intercept [<0.5]	-0.031	0.018
Coefficient of determination [R^2>0.95]	0.995	0.993

Additional testing was performed to demonstrate equivalency of NeuMoDx HBV Quant Assay performance using specimens in primary vs. secondary collection tubes. Panels of HBV negative donor specimens spiked with HBV target (AccuPlex™ HBV Control) were first processed from the primary specimen tubes. The remaining plasma from each specimen was aliquoted into a secondary specimen tube and reprocessed. No significant difference was found in reported results between primary and secondary specimen tube processing.

Equivalency of NeuMoDx HBV Quant Assay performance in fresh vs. frozen serum specimens was also evaluated using a panel of individual, fresh donor serum specimens spiked with HBV at concentrations spanning the linear range of the assay. After processing the fresh specimens, the serum samples were frozen for at least 24 hours at -20 °C. The frozen samples were then thawed and retested. Linear equivalency between identical fresh and frozen samples was evaluated using both Passing-Bablok and Deming Regression Analysis. The Passing-Bablok Regression p-value of 0.329 (greater than 0.05) and Deming Regression Correlation Coefficient of 0.989 show excellent equivalency between samples processed fresh and previously frozen. The bias between the fresh and frozen state was determined by Bland-Altman to be an extremely negligible value of -0.002 $\log_{10}$ IU/mL and further demonstrates the equivalency of fresh vs. frozen specimen processing. Lastly, the correlation between System-reported HBV concentrations and expected concentrations for both fresh and frozen samples was determined by simple linear regression with reported R^2 values of 0.991 and 0.985, respectively.

Specimen Stability

HBV-negative EDTA plasma and serum specimens were spiked with HBV at 3.7 log10 IU/mL and tested at different timepoints while stored onboard the NeuMoDx System – immediately (time 0), after 4 hours, after 8 hours, and after 24 hours. No significant difference in performance was observed across timepoints, indicating that a specimen can be loaded on the NeuMoDx System for up to 24 hours without any impact on assay performance.

Similar testing was also performed with plasma and serum specimens stored in a laboratory refrigerator (between 2 to 8 °C) for up to 7 days prior to testing and no significant difference in performance was observed.

Finally, specimens stored at ≤ -20°C for up to 6 months (plasma) and up to 4 months (serum) before processing were tested and showed no significant difference relative to fresh specimens. The freeze/thaw cycle was repeated and again demonstrated no change in performance after 2 freeze/thaw cycles (plasma) or 4 freeze/thaw cycles (serum).

Method Correlation

Plasma Specimens

Qualitative and quantitative performance of the NeuMoDx HBV Quant Assay were assessed against FDA/CE-approved comparator assays by testing undiluted plasma clinical specimens from HBV infected patients. Testing was performed internally at NeuMoDx through a single-blinded study using clinical specimens obtained from three independent reference laboratories. The results of a total of 308 HBV-positive and negative samples were compiled in the qualitative analysis to calculate the clinical sensitivity and specificity of the NeuMoDx HBV Quant Assay. Qualitative analysis was completed including and excluding the positive samples below LLoQ as classification of such low samples may vary across tests. A total of 97 HBV-positive clinical specimens within the linear range common to both tests were used to generate the linear regression to define the quantitative performance. In addition to providing excellent sensitivity and specificity, the NeuMoDx HBV Quant Test Strip demonstrated excellent quantitative correlation with the comparator assay. Based on these results, the sensitivity of the NeuMoDx HBV Quant Assay was estimated to be 100% (CI 96.4% - 100%) and the specificity was estimated to be 95.6% (CI 91.9%- 97.7%). These 95% Confidence Intervals were calculated using 95% score confidence interval method per EP12-A2, User Protocol for Evaluation of Qualitative Test Performance, Approved Guideline, Vol 28, No 3.[6]

Table 15: Clinical Sensitivity and Specificity Metrics of the NeuMoDx HBV Quant Assay for Plasma Specimens on the NeuMoDx 288 Molecular System

	Reference Assay (POS)	Reference Assay (NEG)	TOTAL
NeuMoDx HBV Quant Assay (POS)	103	9	112
NeuMoDx HBV Quant Assay (NEG)	0	196	196
TOTAL	103	205	308
SENSITIVITY = 100% 95% CI (96.4% - 100%) **SPECIFICITY = 95.6%** 95% CI (91.9% - 97.7%)			

Table 16: Clinical Sensitivity and Specificity Metrics of the NeuMoDx HBV Quant Assay on the NeuMoDx 288 Molecular System with Plasma Samples <LLoQ excluded

	Reference Assay (POS)	Reference Assay (NEG)	TOTAL
NeuMoDx HBV Quant Assay (POS)	99	5	104
NeuMoDx HBV Quant Assay (NEG)	0	196	196
TOTAL	99	201	300
SENSITIVITY = 100% 95% CI (96.3% - 100%) **SPECIFICITY = 97.5%** 95% CI (94.3% - 98.9%)			

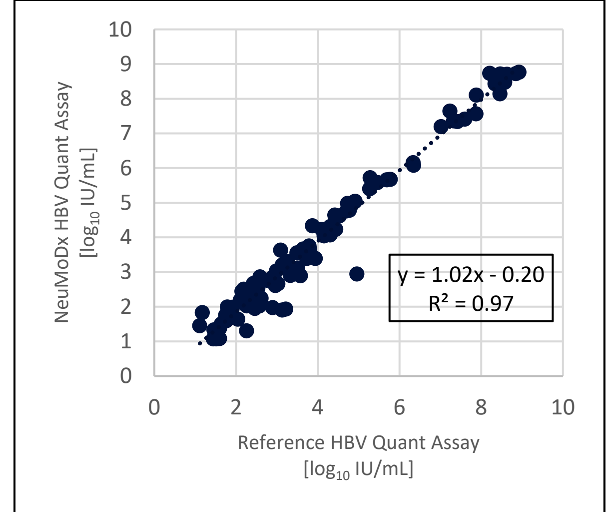

Figure 7: Quantitative Method Correlation Study using the NeuMoDx HBV Quant Assay

Additional testing was performed on the NeuMoDx 96 Molecular System using 159 residual clinical plasma specimens. As with the previous testing performed on the NeuMoDx 288, results obtained from the NeuMoDx 96 were compared to the results reported by FDA approved and/or CE marked assays utilized by source laboratories for the standard of care testing. Results including truth table with clinical Sensitivity and Specificity are presented with 95% CI in *Table 17*.

Table 17: Clinical Performance Summary – NeuMoDx HBV Quant Assay on the NeuMoDx 96 Molecular System

	Reference Assay (POS)	Reference Assay (NEG)	TOTAL
NeuMoDx HBV Quant Assay (POS)	60	2	62
NeuMoDx HBV Quant Assay (NEG)	1	95	96
TOTAL	61	97	158
SENSITIVITY = 98% 95% CI (90% - 100%) **SPECIFICITY = 98%** 95% CI (92% - 100%)			

Serum Specimens

Quantitative performance of the NeuMoDx HBV Quant Assay was assessed against FDA/CE-approved comparator assays by testing de-identified, residual HBV-positive serum specimens from HBV infected patients. A total of 66 clinical known HBV-positive serum specimens obtained from two independent reference laboratories were tested using the NeuMoDx HBV Quant Assay, internally at NeuMoDx. Of the known positive serum specimens tested, 58 were identified as positive results of which nine (9) results were below LLoQ and above ULoQ for the NeuMoDx HBV Quant Assay and/or the reference test. A total of 49 HBV-positive clinical specimens within the linear range common to both tests were used to generate the regression analyses to define the quantitative performance.

Equivalency and residual plots were generated to represent the correlation between the NeuMoDx HBV Quant Assay concentrations and the reference test concentration values for all samples tested using Deming and Passing-Bablok regression analysis, as presented in Figure 8 and 9. The quality of the Deming Regression fit is illustrated by a slope coefficient of 0.99 with a 95% CI (0.93, 1.07), and an intercept (bias) of -0.22 with a 95% CI (-0.56, 0.12), demonstrating that the concentration results obtained between the NeuMoDx HBV Quant Assay and Reference tests are highly correlated and with acceptable bias. The quality of the Passing-Bablok linear fit is illustrated by a slope coefficient of 0.99 with a 95% CI (0.91, 1.06), and an intercept (bias) of -0.25 with a 95% CI (-0.48, 0.06), demonstrating that the concentration results obtained between the NeuMoDx HBV Quant Assay and Reference tests are highly correlated and with acceptable bias as shown in *Table 18*.

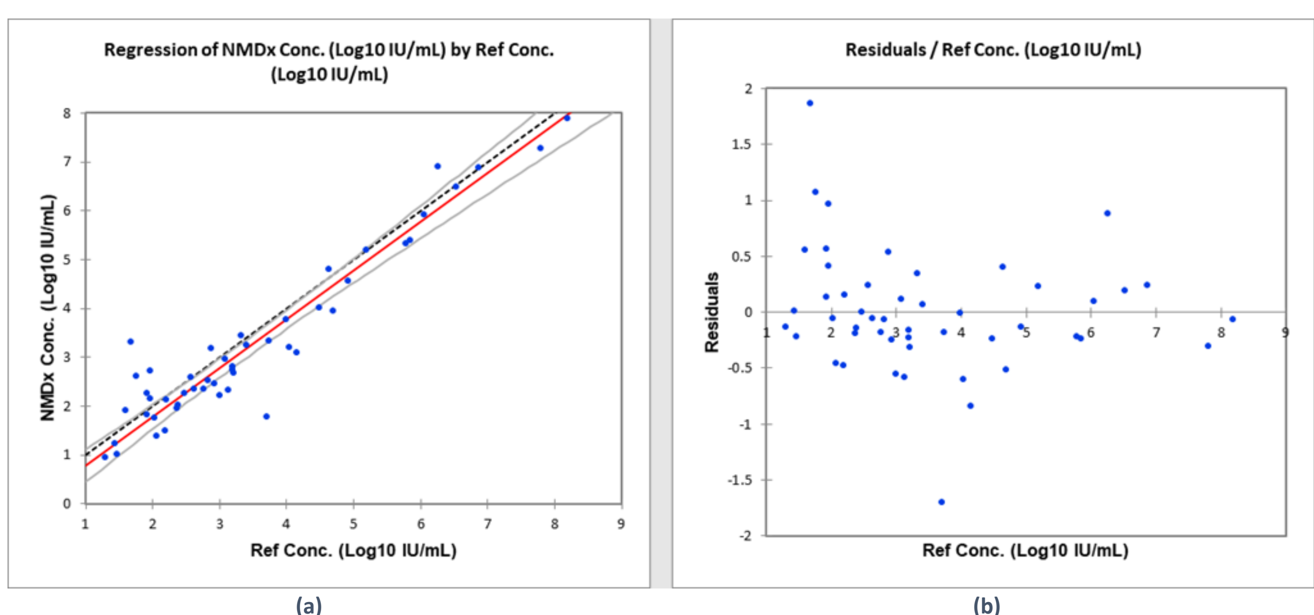

Figure 8: Equivalency (a) and Residual (b) Plots – Cumulative analysis of NeuMoDx HBV Test results vs. Reference tests – Deming Analysis.

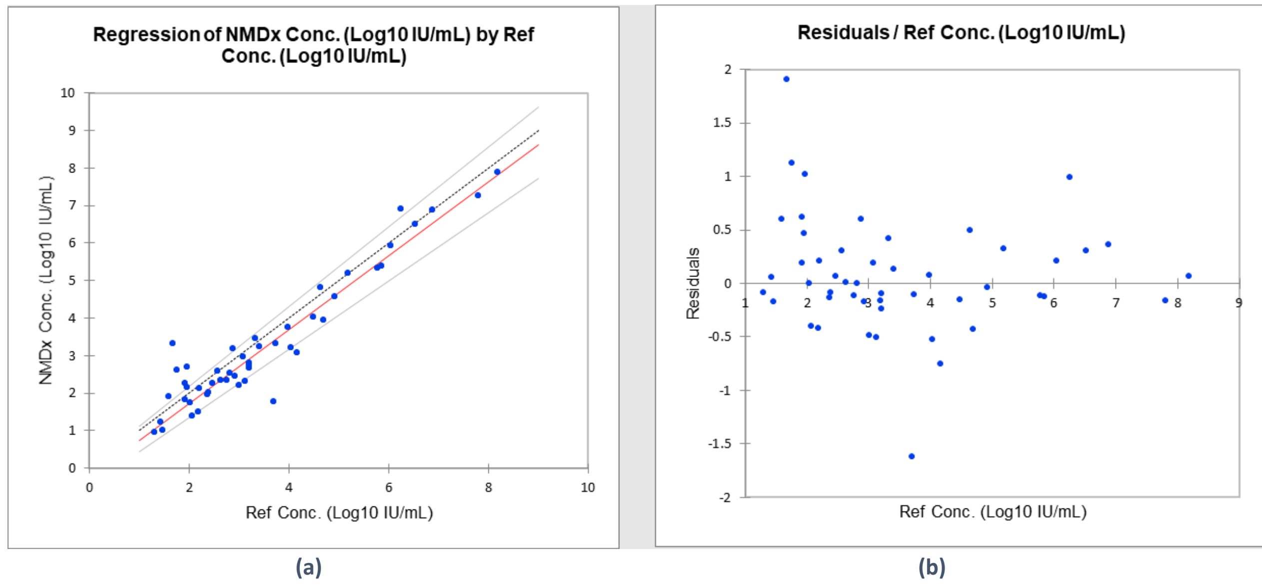

Figure 9: Equivalency (a) and Residual (b) Plots – Cumulative analysis of NeuMoDx HBV Quant Assay results vs. Reference tests – Passing-Bablok Analysis.

Table 18. Summary of Deming and Passing-Bablok Linear Regression Analysis for Serum Specimens

Deming Analysis			Passing-Bablok Analysis		
Intercept	**Slope Coefficient**	**R2**	**Intercept**	**Slope Coefficient**	**p-value**
-0.22 **95% CI (-0.56, 0.12)**	**0.99** **95% CI (0.93, 1.07)**	**0.95**	**-0.25** **95% CI (-0.48, 0.06)**	**0.99** **95% CI (0.91, 1.06)**	**0.89**

Testing of Contrived Specimens – 200 µL Specimen Volume Workflow

Quantitative correlation between the 200 µL and 550 µL specimen volume workflows was confirmed using a panel consisting of individual, HBV-negative plasma and serum samples spiked with four known levels of HBV Control material, traceable to the 4th WHO International Standard for HBV DNA for nucleic acid tests. These individual plasma and serum specimens were processed using both the 550 µL and 200 µL specimen volume workflows for a total of 288 tests performed. Equivalency comparisons between the concentration reported by the NeuMoDx Software for the 200 µL and 550 µL specimen volume workflows with the contrived panel were performed on an individual sample basis. Deming and Passing-Bablok regression analysis had a slope of 0.985 and 0.998 with intercepts of -0.001 and 0.053, respectively in plasma and 1.024 and 1.018 with intercepts of 0.095 and 0.070, respectively in serum, demonstrating excellent concordance of HBV quantifications between the two processing volumes. A Bland and Altman comparison showed a minimal bias between the two workflows. Additionally, simple linear regression analyses with the expected concentration and the reported concentration for the 200 µL workflow had a slope of 1.047 and a correlation coefficient of 0.998 (plasma) and of 1.113 and 0.992 (serum), further supporting excellent performance using the 200 µL specimen volume workflow for the NeuMoDx HBV Quant Assay. Results of these studies are summarized below in *Figure 10* and *Figure 11.*

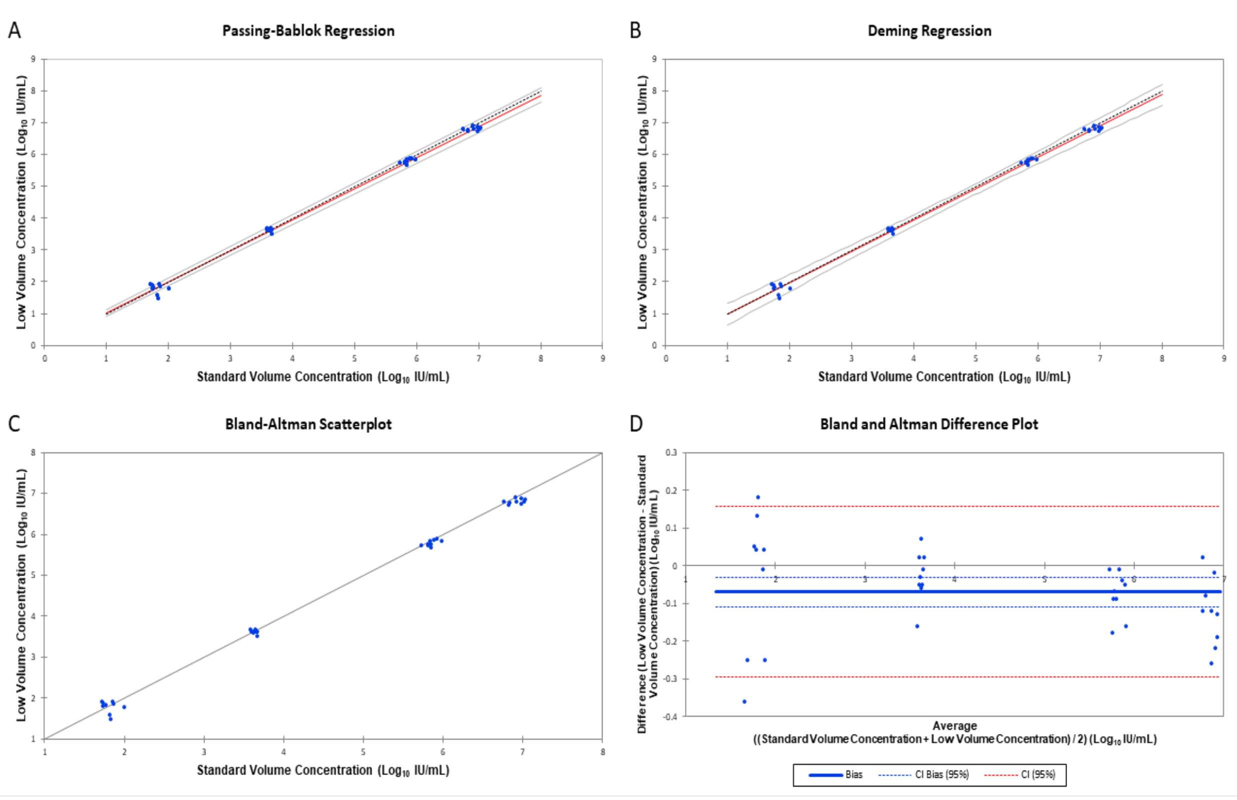

Figure 10: Equivalency Plot Comparisons of Low Volume Reported Concentrations to Standard Specimen Volume Reported Concentrations. A) Passing-Bablok Regression. B) Deming Regression. C) Bland-Altman Scatterplot D) Bland-Altman Difference Plot – Plasma Specimens

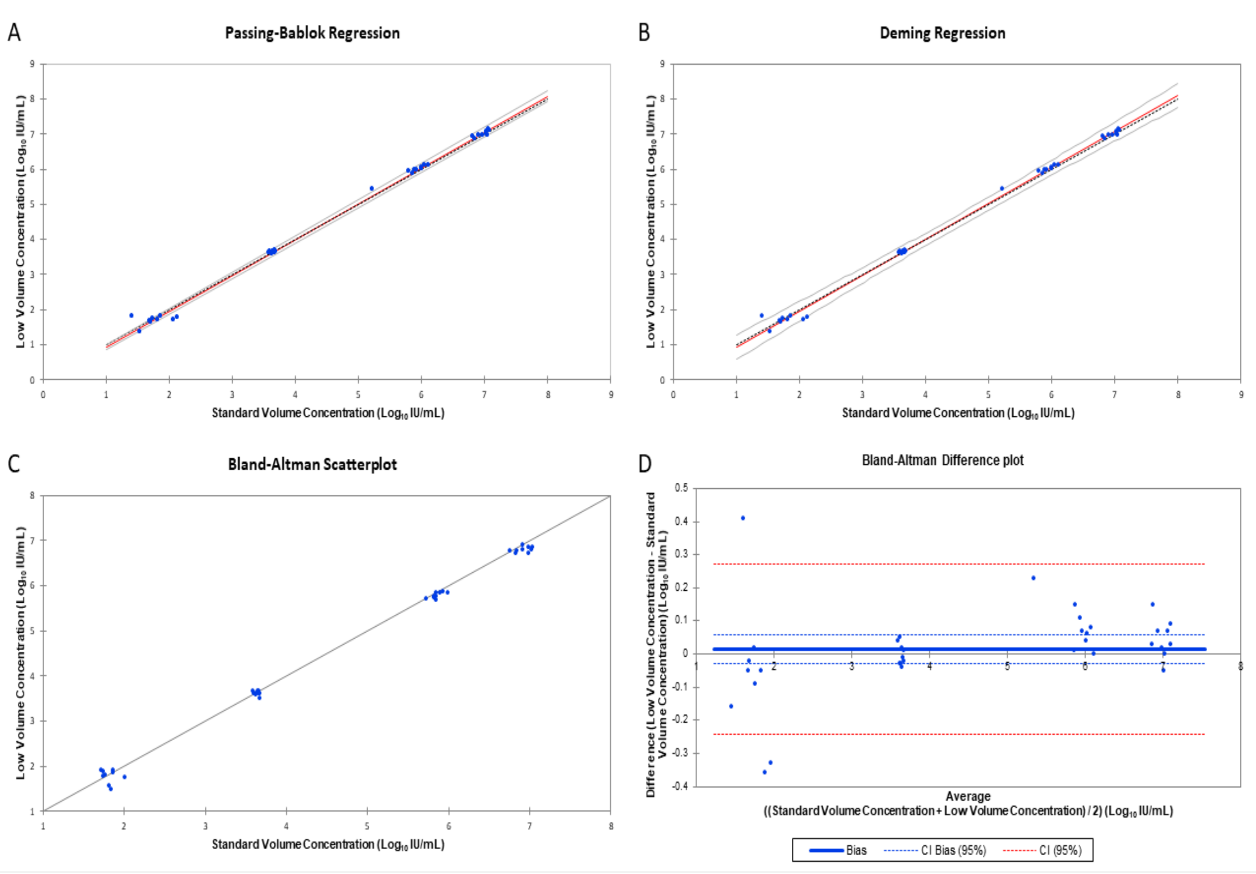

Figure 11: Equivalency Plot Comparisons of Low Volume Reported Concentrations to Standard Specimen Volume Reported Concentrations. A) Passing-Bablok Regression. B) Deming Regression. C) Bland-Altman Scatterplot D) Bland-Altman Difference Plot – Serum Specimens

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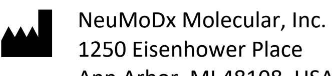
SYMBOL KEY

Symbol	Meaning	Symbol	Meaning
℞ only	Prescription use only		Temperature limit
	Manufacturer		Do not re-use
IVD	*In vitro* diagnostic medical device		Contains sufficient for <*n*> tests
EC REP	Authorized representative in the European Community		Consult instructions for use
REF	Catalog number		Caution
LOT	Batch code		Biological risks
	Use-by date	CE	CE Mark



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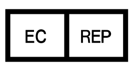

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