REF **300300 NeuMoDx™ HCV 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 HCV Quant Assay is an automated, *in vitro* nucleic acid amplification test for the quantitation of hepatitis C virus (HCV) RNA in human plasma and serum specimens for HCV antibody positive genotypes 1 through 6 of HCV-infected individuals. The NeuMoDx HCV Quant Assay implemented on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx System(s)) incorporates automated RNA extraction to isolate the target nucleic acid from the specimen and real-time reverse transcriptase polymerase chain reaction (RT-PCR) to target the highly conserved sequences in the hepatitis C viral genome.

The NeuMoDx HCV Quant Assay is intended for use as an aid in the management of patients with HCV infections. The results from the NeuMoDx HCV Quant Assay must be interpreted within the context of all relevant clinical and laboratory findings. The NeuMoDx HCV Quant Assay is not intended for use as a screening test for blood or blood products or to diagnose the clinical status of HCV 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 tubes or serum 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 the plasma/serum sample is mixed with NeuMoDx Lysis Buffer 3 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated RNA for real-time RT-PCR amplification, and if present, amplify and detect the products of amplification. The NeuMoDx HCV Quant Assay targets two highly conserved regions of the HCV genome to increase the robustness of the assay. The NeuMoDx HCV Quant Assay also includes an RNA Sample Process Control (SPC2) 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 process.

HCV is a single-stranded, positive sense RNA virus capable of causing both acute and chronic infection.[1] There is currently no vaccine for hepatitis C. While acute infection is usually asymptomatic and very rarely associated with life-threatening disease, more than half of those infected with HCV could develop chronic infection. Of those with chronic HCV infection, the risk of cirrhosis of the liver is between 15-30% within 20 years. Globally an estimated 71 million people are suspected to have chronic HCV infection with a significant number of those expected to develop cirrhosis or liver cancer. [2-4] As a blood borne virus, HCV has been primarily transmitted through blood and blood products. Widespread adoption of blood screening tests has greatly reduced the incidence of infections from donated blood. [1]

Detection of antibodies to HCV does not differentiate between active and cleared infections. Consequently, HCV laboratory testing algorithms require diagnosis of active HCV infections in antibody positive individuals through detection of HCV RNA in plasma or serum prior to initiating therapy (if necessary). Quantitation of HCV RNA (viral load) is now used routinely in defining and monitoring successful HCV treatment.

Current guidelines for the management and treatment of HCV infections recommend quantitative HCV RNA testing before the start of antiviral therapy to establish baseline, and at 12 weeks or later, following the end of treatment. Additional time points may sometimes be recommended. Sustained virologic response (SVR) is the goal of HCV therapy and is defined as undetectable HCV RNA (with an assay that has a limit of detection of < 25 IU/mL) after therapy.[5-7] Recent guidelines from the American Association for the Study of Liver Diseases suggest testing HCV RNA not only at baseline, but also periodically during treatment (i.e., 4 weeks) and at 12 weeks following completion of treatment. Tests for detection of HCV RNA, in combination with the serological tests, are used to identify an active HCV infection.[6]

PRINCIPLES OF THE PROCEDURE

The NeuMoDx HCV Quant Assay combines automated RNA extraction, amplification, and detection by real-time RT-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 3 and the agents contained in the NeuMoDx Extraction Plate to begin processing. The NeuMoDx System automates and integrates RNA extraction and concentration, reagent preparation, and nucleic acid amplification/detection of the target sequences using real-time RT-PCR. The included Sample Process Control (SPC2) 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, RNA 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 RNA is then eluted using

NeuMoDx Release Reagent. The NeuMoDx System uses the eluted RNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the HCV and SPC2 targets. This enables simultaneous amplification and detection of both target and control RNA sequences. Upon reconstitution of the dried RT-PCR reagents, the NeuMoDx System dispenses the prepared RT-PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Reverse transcription, amplification, and detection of the control and target 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 a 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 RT-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 HCV RNA. For detection of the SPC2, 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
300300	**NeuMoDx HCV Quant Test Strip** *Dried RT-PCR reagents containing HCV and SPC2 specific TaqMan probes 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*
800200 or 800202	**NeuMoDx HCV Calibrators** *Single use sets of HCV High and Low Calibrators to establish validity of calibration curve*
900201 or 900202	**NeuMoDx HCV External Controls** *Single use sets of HCV Positive and Negative Controls*
400600	**NeuMoDx Lysis Buffer 3**
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 HCV 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 HCV Calibrators) must be available before test results can be generated for clinical samples.
- NeuMoDx HCV External Controls must be processed every 24 hours throughout testing with the NeuMoDx HCV Quant Assay.
- Minimum specimen volume of secondary aliquots is dependent on the tube size, specimen tube carrier, and specimen volume processing as defined below. Volume lower than 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 ribonuclease (RNase) contamination of all reagents and consumables at all times. The use of sterile RNase-free disposable transferring pipettes is recommended if using secondary specimen 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 HCV 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 HCV Quant Test Strip and NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 3; 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* [8] and in CLSI Document M29-A4. [9]
- 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 HCV 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 HCV Quant Test Strip may remain onboard the NeuMoDx System for up to 14 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 or in plasma preparation tubes (PPT). Follow the specimen collection tube manufacturer instructions for preparation and storage.
4. To prepare serum specimens, whole blood should be collected in serum tubes or 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 prior to processing. If additional storage time is required, it is recommended that the specimens be either refrigerated or frozen in 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 at room temperature.
8. Prepared specimens in secondary tubes may be stored at ≤ -20 °C for up to 24 weeks before processing; frozen specimens should not undergo more than two (2) freeze/thaw cycles prior to use.

a. Plasma specimens that are frozen and undergo one (1) freeze-thaw cycle can be stored onboard the system for additional 8 hours.
b. Plasma specimens that are frozen and undergo two (2) freeze-thaw cycles should not be stored onboard the system for more than 4 hours.
c. Serum Specimens that are frozen and undergo one (1) or two (2) freeze-thaw cycles shall be tested immediately after thawing.
d. If samples are frozen, allow the samples to completely thaw at room temperature (15–30 °C); vortex to generate a uniformly distributed sample.
e. 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 HCV testing.
11. Proceed to *Test Preparation* section.

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

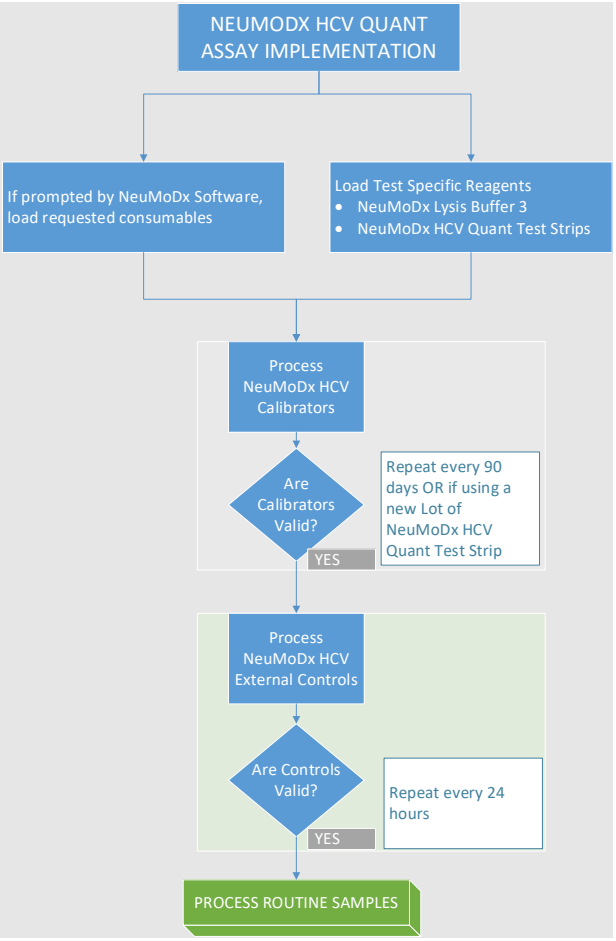

Figure 1: NeuMoDx HCV Quant Assay Implementation Workflow

INSTRUCTIONS FOR USE

Test Preparation

The NeuMoDx HCV 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 volume processing workflows—550 µL specimen volume workflow or 200 µL specimen volume workflow.

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 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*** buffy coat 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:
   a. Gently vortex the specimen to achieve uniform distribution
   b. Using a new transfer pipette for each specimen, transfer an aliquot of the plasma or 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

   c. Care should be taken not to transfer any clots from the sample into the specimen tube.

NeuMoDx System 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 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 HCV 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 HCV Calibrators and/or NeuMoDx HCV 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 HCV Quant Test Strip can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx HCV Quant Test Strip has been established for plasma specimens prepared with EDTA/ACD as anti-coagulant or serum specimens prepared in serum separator tubes. Use of the NeuMoDx HCV 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 HCV Quant Test Strip has been established for primary tube testing using BD Vacutainer Plus Plastic K_2EDTA Tubes, BD Vacutainer PPT Plasma Preparation Tubes, BD Vacutainer Plus Plastic Serum Tubes, and BD Vacutainer SST Tubes.
4. Specimen handling beyond the storage conditions can negatively impact the quantitative accuracy of the NeuMoDx HCV Quant Assay but less likely to impact the qualitative (Positive/Negative) call rate.
5. Storing serum specimens onboard the system after prolonged frozen storage and undergoing two freeze-thaw cycles without immediate testing can negatively impact the quantitative accuracy of the NeuMoDx HCV Quant Assay.
6. A small increase in the limit of detection and lower limit of quantitation of the NeuMoDx HCV Quant Assay has been observed when using the 200 µL specimen volume workflow.
7. The NeuMoDx HCV Quant Assay must not be used with samples from heparinized humans.
8. Since detection of HCV is dependent on the number of target RNA viral particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
9. NeuMoDx HCV Calibrators and NeuMoDx HCV External Controls must be processed as recommended in the package inserts when prompted by NeuMoDx System software before processing routine clinical samples.
10. 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 HCV Quant Assay.
11. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
12. If both the HCV target and the SPC2 target do not amplify, an invalid result (Indeterminate, No Result, or Unresolved) will be reported and the test should be repeated.
13. If the NeuMoDx HCV Quant Assay result is Positive, but the quantitation value is beyond the limits of quantitation, the NeuMoDx System will report whether the detected HCV was *below* Lower Limit of Quantitation (LLoQ) or *above* Upper Limit of Quantitation (ULoQ).
14. In the event the detected HCV is *below* LLoQ, the NeuMoDx HCV Quant Assay may be repeated (if desired) with another aliquot of the specimen.
15. In the event the detected HCV is above ULoQ, the NeuMoDx HCV Quant Assay may be repeated with a diluted aliquot of the original specimen. A 1:100 or 1:1000 dilution in HCV negative plasma or Basematrix 53 Diluent (Basematrix) (SeraCare, Milford, MA) is recommended. The concentration of the original specimen is calculated as follows:

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

16. The occasional presence of PCR inhibitors in plasma and serum 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.
17. A positive result does not necessarily indicate the presence of viable organisms. However, a positive result is presumptive for the presence of hepatitis C virus RNA.
18. Deletion or mutations in the conserved regions targeted by the NeuMoDx HCV Quant Assay may affect detection or could lead to an erroneous result using the NeuMoDx HCV Quant Test Strip.
19. Results from NeuMoDx HCV Quant Assay should be used as an adjunct to clinical observations and other information available to the physician; the test is not intended to diagnose infection.
20. 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 HCV Quant Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx HCV Assay Definition File (HCV ADF). A result may be reported as Negative, Positive with a reported HCV 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 NeuMoDx HCV Quant Assay Decision Algorithm

RESULT	HCV Target	Sample Process Control (SPC2)	Result Interpretation
Positive with Reported Concentration	Amplified 0.9 ≤ [HCV] ≤ 8.2 $\log_{10}$ IU/mL (550 µL Workflow) 1.5 ≤ [HCV] ≤ 8.2 $\log_{10}$ IU/mL (200 µL Workflow)	Amplified or Not Amplified	HCV RNA detected within quantitative range
Positive, above ULoQ	Amplified [HCV] > 8.2 $\log_{10}$ IU/mL	Amplified or Not Amplified	HCV RNA detected above quantitative range
Positive, below LLoQ	Amplified [HCV] < 0.9 $\log_{10}$ IU/mL (550 µL Workflow) [HCV] < 1.5 $\log_{10}$ IU/mL (200 µL Workflow)	Amplified or Not Amplified	HCV RNA detected below quantitative range
Negative	Not Amplified	Amplified	HCV RNA 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 HCV Quant Assay, the concentration of HCV RNA 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 HCV Calibrators processed to establish validity of the standard curve for a given lot of the NeuMoDx HCV Quant Test Strip on a specific NeuMoDx System.
    b. The calibration coefficient is incorporated into the final determination of the concentration of HCV RNA.
    c. The NeuMoDx Software accounts for the specimen input volume when determining the concentration of HCV RNA per mL of specimen.
2. NeuMoDx HCV Quant Assay results are reported in $\log_{10}$ IU/mL.
3. The resulting quantitation of the unknown samples is traceable to the WHO 5th HCV International Standard.

Test Calibration

A valid calibration based on the Standard Curve is required to quantitate HCV RNA 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 HCV Calibrators needs to be processed with each new lot of NeuMoDx HCV Quant Test Strips, if a new HCV 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 $\log_{10}$ IU/mL and the high calibrator nominal target is 5 $\log_{10}$ 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 HCV 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 HCV 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 HCV Positive result and the negative control should provide an HCV 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 HCV External Controls with fresh vials of the control(s) failing the validity test.
   d) If positive NeuMoDx HCV external control continues to report a Negative result, contact NeuMoDx technical service.
   e) If negative NeuMoDx HCV 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 (SPC2) is incorporated in the NeuMoDx Extraction Plate and undergoes the entire process of nucleic acid extraction and real-time RT-PCR amplification with each sample. Primers and probe specific for SPC2 are also included in each NeuMoDx HCV Quant Test Strip enabling detection of presence of SPC2 along with the target HCV RNA (if present) via multiplex real-time RT-PCR. Detection of SPC2 amplification allows the NeuMoDx System software to monitor the efficacy of the RNA extraction and RT-PCR amplification processes.

Invalid Results

If a NeuMoDx HCV 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 HCV RNA or SPC2 is detected, in the absence of system errors, which indicates possible reagent failure or the presence of inhibitors. In the event a UNR result is reported, a retest is recommended as a first step. If a retest fails, a diluted specimen may be used to mitigate the effects of any sample inhibition.

If a NeuMoDx HCV 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 HCV Quant Assay was characterized by testing negative specimens and a dilution series of the WHO 5th International Standard (genotype 1) 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 study was performed over 3 days across multiple systems with multiple lots of NeuMoDx reagents. Each system (N288 and N96) processed 18 replicates at each dilution level per day. Detection rates are depicted in *Table 2*. An additional study was executed to determine the LoD of the NeuMoDx HCV Quant Assay when using the 200 µL specimen volume workflow, the results of which are shown in *Table 3.*

Table 2. Positive Detection Rates for LoD Determination of the NeuMoDx HCV Quant Assay – 550 µL Workflow

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
30	1.48	108	108	100%	108	108	100%
15	1.18	108	108	100%	108	107	99%
10	1.00	108	105	97%	108	102	94%
7.5	0.88	108	102	94%	108	105	97%
3.75	0.57	108	84	78%	108	86	80%
1.875	0.27	108	47	44%	108	63	58%
NEG	0	108	0	0%	107	1	0.93%

Table 3. Positive Detection Rates for LoD Determination of the NeuMoDx HCV Quant Assay – 200 µL Workflow

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
75	1.88	N/A	N/A	N/A	22	22	100%
60	1.78	22	22	100%	22	22	100%
30	1.48	22	21	95.5%	22	20	90.9%
15	1.18	22	17	77.3%	22	19	86.4%
10	1.00	22	13	59.1%	22	15	68.2%
NEG	0	22	0	0%	22	0	0%

The LoD of the NeuMoDx HCV Quant Assay in plasma across all genotypes was determined to be 7.5 IU/mL (95% CI 6.4 to 9.2 IU/mL) [(0.9 Log_{10} IU/mL) (95% CI 0.8 to 1.0 log_{10} IU/mL)] as tested on the NeuMoDx 288 Molecular System using the 550 µL specimen volume workflow (*Figure 2)*. The LoD of the NeuMoDx HCV Quant Assay for serum specimens was determined to be 8.0 IU/mL (95% CI 6.6 to 10.4 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*); the LoD claim for both specimen types using the 550 µL specimen volume workflow is **8.0 IU/mL (0.9 log_{10} IU/mL**.

The LoD of the NeuMoDx HCV Quant Assay using the 200 µL specimen volume workflow was found to be 27.9 IU/mL (95% CI 20.1–81.9) in plasma specimens and 29.8 IU/mL (95%CI 20.5–94.0) in serum specimens (*Figure 3)*; the LoD claim for both specimen types using the 200 µL specimen volume workflow is **30.0 IU/mL (1.5 log_{10} IU/mL)**.

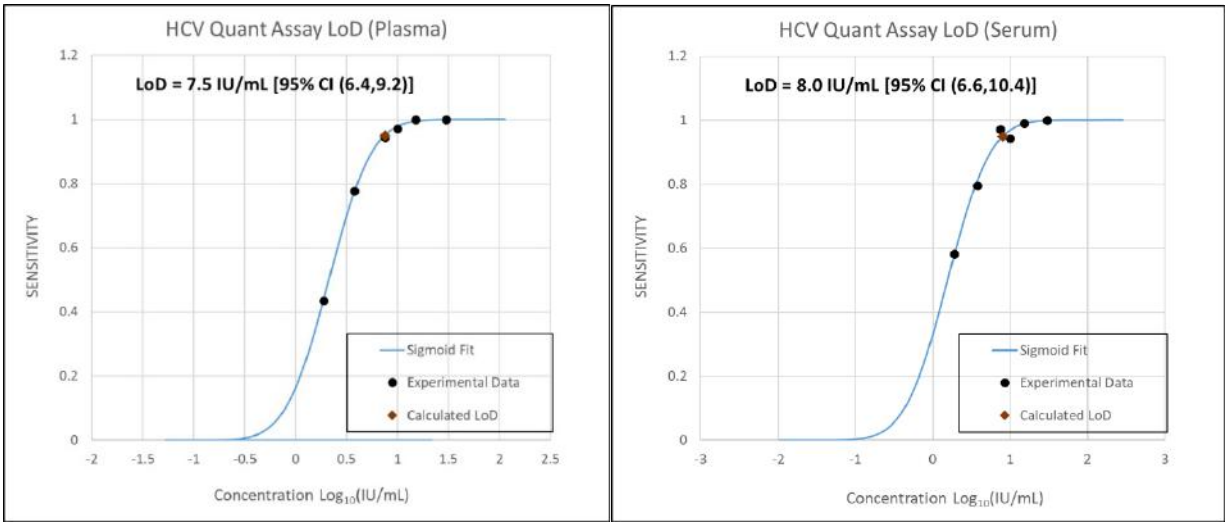

Figure 2: Probit Style Analysis Used to Determine the LoD of the NeuMoDx HCV Quant Assay, Plasma (left) and Serum (right) – 550 µL Workflow

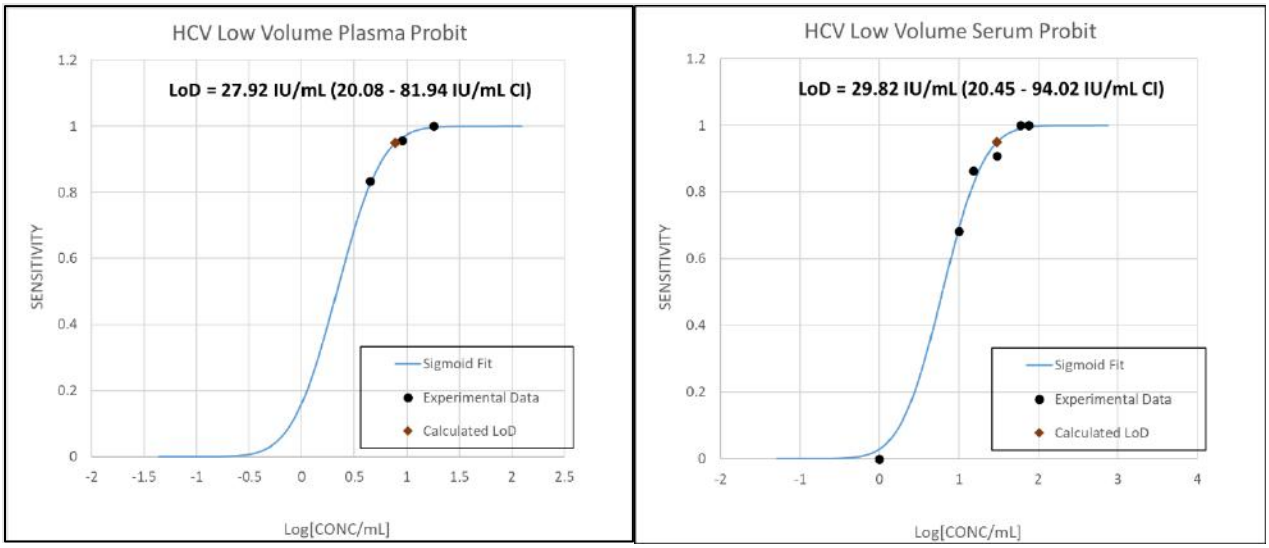

Figure 3: Probit Style Analysis Used to Determine the LoD of the NeuMoDx HCV Quant Assay, Plasma (left) and Serum (right) – 200 µL Workflow

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 HCV 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 6 levels of HCV plasma and serum specimens tested in the LLoQ study using genotype 1 with the 550 µL specimen volume workflow are shown in *Table 4.* Results from additional testing using the 200 µL specimen volume workflow are shown in *Table 5.*

Table 4. NeuMoDx HCV Quant Assay LLoQ, with the Bias and TAE – 550 µL Workflow

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
30.00	1.48	1.41	100	0.32	0.07	0.71	1.39	100	0.30	0.08	0.69
15.00	1.18	1.24	100	0.36	0.06	0.79	1.23	99	0.32	0.06	0.70
10.00	1.00	1.07	97	0.35	0.07	0.77	1.14	94	0.36	0.14	0.85
7.50	0.88	1.01	94	0.44	0.13	1.02	1.12	97	0.25	0.25	1.09
3.75	0.57	1.08	78	0.43	0.51	1.38	1.17	80	0.58	0.59	1.76
1.88	0.27	1.11	44	0.36	0.83	1.55	1.11	58	0.69	0.84	2.22

Table 5. NeuMoDx HCV Quant Assay LLoQ, with the Bias and TAE – 200 µL Workflow

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
75	1.88	N/A	N/A	N/A	N/A	N/A	1.56	100	0.23	0.32	0.78
60	1.78	1.93	100	0.39	0.15	0.93	1.56	100	0.27	0.22	0.76
30	1.48	1.35	96	0.44	0.11	0.99	1.45	91	0.41	0.03	0.85
15	1.18	1.37	77	0.42	0.18	1.03	1.36	86	0.53	0.18	1.25
10	1.00	1.26	59	0.56	0.25	1.36	1.15	68	0.53	0.15	1.21

The LLoQ for the NeuMoDx HCV Quant Assay is determined to be 7.7 IU/mL (0.9 log10 IU/mL) for plasma, and 8.4 IU/mL, (0.9 log_{10} IU/mL) for serum using the 550 µL specimen volume workflow; the LLoQ for both plasma and serum is determined to be **8.4 IU/mL (0.9 log_{10} IU/mL)** using the 550 µL specimen volume workflow.

The LLoQ for the NeuMoDx HCV Quant Assay using the WHO Standard is determined to be 30.0 IU/mL (1.5 log_{10} IU/mL) for plasma, and 29.8 IU/mL, (1.37 log_{10} IU/mL) for serum using the 200 µL specimen volume workflow; the LLoQ for both plasma and serum is determined to be **30.0 IU/mL (0.9 log_{10} IU/mL)** using the 200 µL specimen volume workflow.

Analytical Sensitivity - LoD and LLoQ across HCV Genotypes

The LoD was initially established for Genotype 1 (5th WHO International Standard) and then additional testing was performed around the established LoD using each of the other 5 genotypes. Thirty-six (36) replicates at levels corresponding to 2X, 1X and 0.5X of the 95% CI upper limit of LoD were tested using the NeuMoDx HCV 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. These results confirm that the NeuMoDx HCV Quant Assay has excellent and equivalent detection performance across all six genotypes with a range of 4.5 – 7.5 IU/mL, including the results obtained with the 5th WHO International Standard (Genotype 1). Overall, the LoD of the NeuMoDx HCV Quant Assay across genotypes was determined to be 7.5 IU/mL (0.88 Log_{10} IU/mL) and the LLoQ was determined to be the highest value which is 7.7 IU/mL (0.9 Log_{10} IU/mL), as was reported for the 5th WHO International Standard (Genotype 1, above). *Table 6* shows the LoD and LLoQ results for testing across HCV genotypes as determined in plasma.

Table 6. HCV Genotypes Tested in Plasma using 550 µL Specimen Volume Workflow

GENOTYPE	LoD [IU/mL]	LLoQ [IU/mL]
1	**7.5**	**7.7**
2	4.5	5.2
3	7.5	7.5
4	6.0	6.0
5	4.8	5.5
6	4.5	6.7

Based on the outcome of the above referenced studies, NeuMoDx claims an **LoD** of **8.0 IU/mL (0.9 log_{10} IU/mL)** and an **LLoQ of 8.4 IU/mL (0.9 log_{10} IU/mL)** for the NeuMoDx HCV Quant Assay in ***plasma and serum*** using the **550 µL specimen volume workflow**.

The claimed **LoD and LLoQ** for the NeuMoDx HCV Quant Assay for **both specimen types (*plasma and serum*)** using **the 200 µL specimen volume workflow is 30.0 IU/mL (1.5 log_{10} IU/mL).**

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

Linearity and the Upper Limit of Quantitation (ULoQ) of the NeuMoDx HCV Quant Assay were established in plasma by preparing a dilution series using HCV Armored RNA® (Asuragen Inc., Austin, TX) and AcroMetrix™ High Control HCV (Thermo Fisher Scientific, Waltham, MA) with established traceability to the 5th WHO International Standard. An 11-member panel was prepared in pooled HCV-negative plasma to create a panel that would span a concentration range of 8.2-1.5 log_{10} IU/mL. The NeuMoDx HCV Quant Assay demonstrated the ability to quantify HCV across the 8 log_{10} linear range with an accuracy of ±0.3 log_{10} IU/mL based on the Standard Error as calculated by the 95% confidence interval. No significant benefit was gained using 2nd and 3rd order regression fits. The ULoQ in plasma was determined to be 8.2 log_{10} IU/mL. A subsequent study was performed to demonstrate matrix equivalency and the analysis compared the NeuMoDx HCV 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 R2 value of 0.99 (MS Excel Regression Tool) or a p-value of 0.600 (Passing-Bablok). The HCV assay concentrations reported by the NeuMoDx System compared to the expected values are presented in *Figure 4.*

Linearity and ULoQ were then evaluated with the 200 µL specimen volume workflow. 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 HCV across the linear range of the NeuMoDx HCV Quant Assay using the 200 µL specimen volume workflow.

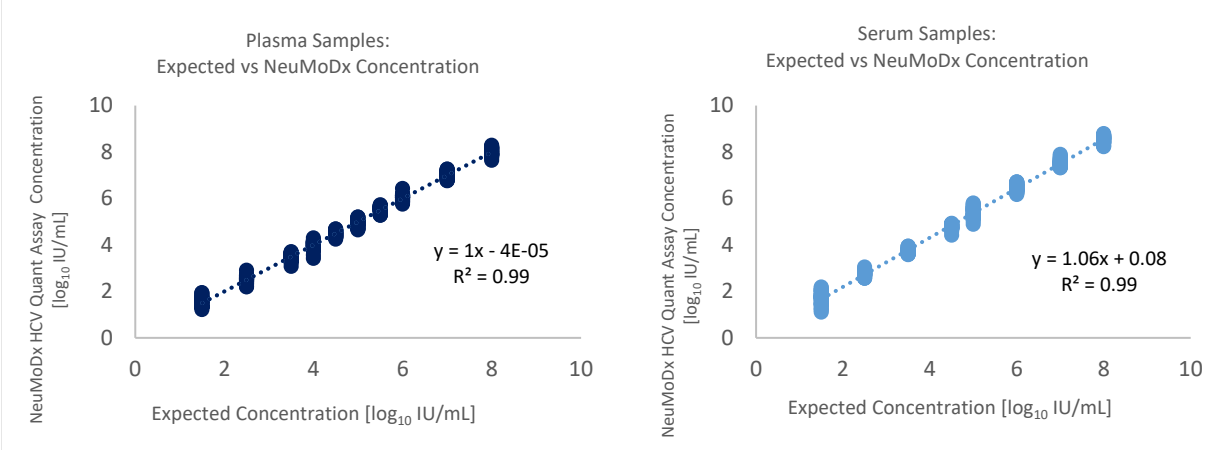

Figure 4: Linear range of the NeuMoDx HCV Quant Assay Plasma (left) and Serum (right) – 550 µL Workflow

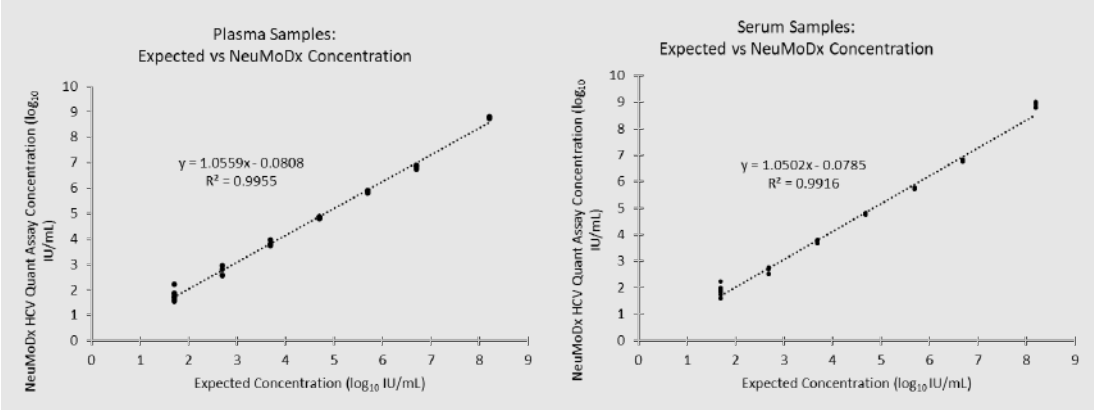

Figure 5: Linear range of the NeuMoDx HCV Quant Assay Plasma (left) and Serum (right) – 200 µL Workflow

Analytical Sensitivity – Linearity Across Genotypes

The linearity of the NeuMoDx HCV Quant Assay across six HCV genotypes was characterized by testing at least four (4) different concentrations of each genotype of HCV prepared in pooled HCV-negative plasma. The levels of HCV targets tested 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. The linearity across six HCV genotypes are presented in *Table 7* and *Figure 6*.

Table 7. Linearity of the NeuMoDx HCV Quant Assay Across Genotypes

Genotype	Linearity Equation y = NeuMoDx HCV Assay Quantitation x = Expected Quantitation	R^2
1	y = 1.054x + 0.1325	0.979
2	y = 1.0792x - 0.0748	0.985
3	y = 1.0423x - 0.0439	0.981
4	y = 1.0158x + 0.0292	0.973
5	y = 0.9873x + 0.1524	0.994
6	y = 1.0393x + 0.0396	0.997

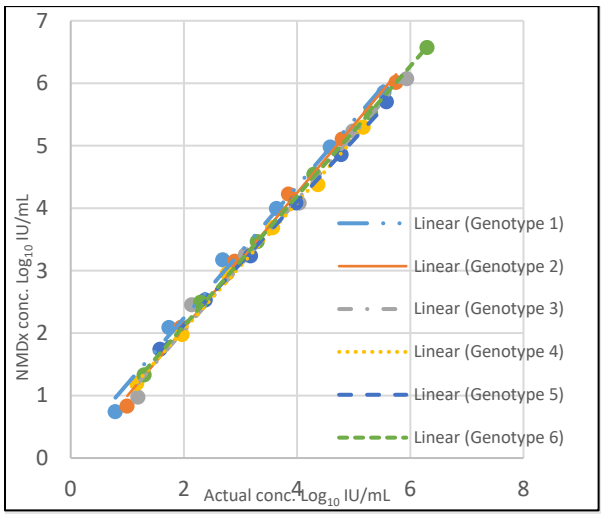

Figure 6: Linearity of the NeuMoDx HCV Quant Assay across Genotypes

Analytical Specificity – Cross-Reactivity

Analytical specificity was demonstrated by screening 33 organisms commonly found in blood/plasma specimens as well as species phylogenetically similar to HCV 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 8*. No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx HCV Quant Assay.

Table 8. Pathogens Used to Demonstrate Analytical Specificity

Non-Target Organisms						
Adenovirus 2	Dengue V1	Hepatitis A	Human Immunodeficiency Virus-2	Human T-lymphotropic virus 1	Propionibacterium acnes	West Nile virus
Adenovirus 5	Dengue V2	Hepatitis B	Human papillomavirus 16	Human T-lymphotropic virus 2	Rubella	Yellow Fever
Candida albicans	Dengue V3	Herpes simplex virus (HSV) 1	Human papillomavirus 18	Influenza A	St. Louis encephalitis	Zika virus
Chlamydia trachomatis	Dengue V4	Herpes simplex virus (HSV) 2	Human Herpes virus 6b	Neisseria gonorrhoeae	Staphylococcus aureus	
Cytomegalovirus	Epstein-barr virus	Human Immunodeficiency Virus-1	Human Herpes virus 8	Parvovirus B19	Staphylococcus epidermidis	

Analytical Specificity – Interfering Substances, Commensal Organisms

The NeuMoDx HCV Quant Assay was evaluated for interference in the presence of non-target organisms using the same organism pools prepared for the cross-reactivity testing listed above in *Table 8.* Negative HCV plasma was spiked with the organisms pooled in groups of 4-6, and also spiked with an HCV positive control at a concentration of 1.4 $\log_{10}$ 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.

Analytical Specificity – Interfering Substances, Endogenous and Exogenous Substances

The NeuMoDx HCV Quant Assay was evaluated in the presence of typical exogenous and endogenous interfering substances encountered in HCV clinical plasma specimens. These included abnormally high levels of blood components as well as common antiviral medications, which were classified in *Table 9*. Each substance was added to screened HCV-negative human plasma spiked with 1.7 $\log_{10}$ IU/mL HCV and samples were analyzed for interference. In addition, common disease state plasma associated with hepatitis C infection were also tested for potential interference. The average concentration and bias of all substances tested are reported in *Table 10*. None of the exogenous and endogenous substances affected the specificity of the NeuMoDx HCV Quant Assay.

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

	Product	Classification		Product	Classification
Pool 1	Sofosbuvir	Direct Acting HCV Antiviral	Pool 2	Paritaprevir	HCV NS3/4A Protease Inhibitor
	Ledipasvir	HCV inhibitor		Ombitasvir	HCV Antiviral
	Velpatasvir	HCV NS5A Inhibitor		Ritonavir	HIV Protease Inhibitor
	Clarithromycin	Antibiotic		Abacavir sulfate	Reverse Transcriptase Inhibitor
	Interferon alfa-2a	Immune Modulator		Ribavirin	Immune Modulator
Pool 3	Grazoprevir	HCV NS3/4A Protease Inhibitor	Pool 4	Efavirenz	Reverse Transcriptase Inhibitor
	Elbasvir	HCV NS5A Inhibitor		Lopinavir	Protease Inhibitor
	Tenofovir disoproxil	HBV/HIV Antiviral		Azithromycin	Antibiotic
	Lamivudine	HBV/HIV Antiviral		Dolutegravir	HIV Antiviral
	Valganciclovir	CMV Antiviral		Simeprevir	HCV NS3/4A Protease Inhibitor
Pool 5	Emtricitabine	HIV Antiviral			
	Raltegravir	HIV Antiviral			
	Amoxicillin	Antibiotic			
	Rilpivirine	HIV Antiviral			
	Dasabuvir	HCV Direct Acting Antiviral			
	Glecaprevir	HCV NS3/4A Protease Inhibitor			

Table 10. Interference Testing - Exogenous and Endogenous Agents

Endogenous	**Average Conc. log_{10} IU/mL**	**Bias log_{10} IU/mL**
Hemoglobin	1.61	0.28
Triglycerides	1.31	-0.02
Bilirubin	1.47	0.14
Albumin	1.47	0.14
Exogenous (Medications)	**Average Conc. log_{10} IU/mL**	**Bias log_{10} IU/mL**
Pool 1: Zidovudine (ZDV), Saquinavir, Ritonavir, Clarithromycin, Interferon alfa-2a, Interferon alfa-2b	1.48	0.15
Pool 2: Abacavir sulfate, Amprenavir, Ribavirin, Entecavir, Fluoxetine, Valacyclovir Hydrochloride	1.40	0.07
Pool 3: Tenofovir disoproxil, Lamivudine, Ganciclovir, Valganciclovir, Nevirapine	1.40	0.07
Pool 4: Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin, Paroxetine,	1.51	0.18
Pool 5: Adefovir (dipivoxil), Azithromycin, Indinavir sulfate, Sertaline	1.40	0.07
Disease State	**Average Conc. log_{10} IU/mL**	**Bias log_{10} IU/mL**
Antinuclear Antibody (ANA)	1.53	0.18
Systemic Lupus Erythematosus (SLE)	1.29	-0.06
Rheumatoid Arthritis	1.39	0.04
HBV Antibodies	1.45	0.10
Alcoholic cirrhosis	1.43	0.08
Rheumatoid Factor	1.43	0.08
Non-Alcoholic Steatohepatitis (NASH)	1.32	-0.03

Within Lab Precision

Precision of the NeuMoDx HCV Quant Assay was determined by testing a 7-member panel of HCV specimens prepared (incorporating both HCV Armored RNA and AcroMetrix HCV Control) 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 ≤ 0.26 $\log_{10}$ IU/mL. No significant difference was found in performance across systems, days, or runs as shown in *Table 11*. Precision between operators was not characterized as the operator plays no significant role in the processing of samples using the NeuMoDx System.

Table 11. Within Lab Precision – NeuMoDx HCV Quant Assay on NeuMoDx Systems

	Target Conc. [log10 IU/mL]	Avg Conc. [log10 IU/mL]	Within System SD	Within Day SD	Within Run SD	Within Lab (Overall) SD
ARMORED	6	5.95	0.17	0.13	0.10	0.17
	5	4.87	0.20	0.14	0.12	0.20
	3	2.89	0.19	0.17	0.17	0.19
ACROMETRIX	4.4	4.45	0.12	0.10	0.08	0.13
	3.4	3.45	0.12	0.12	0.11	0.13
	2.4	2.41	0.17	0.15	0.15	0.17
	1.4	1.40	0.26	0.25	0.25	0.24

Lot to Lot Reproducibility

Lot to Lot Reproducibility of the NeuMoDx HCV Quant Assay was determined using three different lots of key reagents – NeuMoDx Lysis Buffer 3, NeuMoDx Extraction Plates and the NeuMoDx HCV Quant Test Strips. A 7-member panel of HCV (incorporating both HCV Armored RNA and AcroMetrix HCV Control) was used to assess performance. Testing was performed using the three lots of reagents on three systems across 6 days. The variation within and across lots was analyzed and results presented in *Table 12*. Maximum overall bias was 0.24 $\log_{10}$ IU/mL and maximum overall SD was 0.33 $\log_{10}$ IU/mL. No significant difference was found in performance across lots as quantitation of all panel members was within tolerance specification.

Table 12. Lot to Lot Reproducibility – NeuMoDx HCV Quant Assay

	Target Conc. [$\log_{10}$ IU/mL]	Mean Conc. OVERALL [$\log_{10}$ IU/mL]	n (Valid Results Per Lot)	ABS BIAS	Between Lots SD	Within Lot SD	Overall SD
ARMORED	6	5.76	36	0.24	0.35	0.13	0.37
	5	4.84	36	0.16	0.16	0.22	0.27
	3	2.81	36	0.19	0.31	0.16	0.35
ACROMETRIX	4.4	4.35	36	0.05	0.21	0.11	0.24
	3.4	3.31	36	0.09	0.17	0.11	0.20
	2.4	2.33	36	0.07	0.24	0.13	0.27
	1.4	1.38	36	0.02	0.23	0.13	0.33

Effectiveness of Control

The SPC2 is included in the NeuMoDx HCV Quant Assay to report process step failures or inhibition affecting performance of the assay. The efficacy was tested under conditions representative of critical process step failures that could potentially occur during sample processing which *may not be detected* by the NeuMoDx System performance monitoring sensors. Positive (3 $\log_{10}$ IU/mL) and negative specimens were challenged in the presence of a control under the following conditions: presence of inhibitor, no wash reagent delivered, and no wash blow out. Process inefficiencies that had an adverse effect on HCV detection/quantitation were mirrored by performance of SPC2 target as shown in *Table 13*. In all instances tested, it was demonstrated that either the sample process control monitored the process inefficiencies and presence of inhibitors adequately or the anticipated process inefficiency did not have a significant adverse effect on SPC2 detection nor HCV detection and quantitation. Therefore, the SPC2 demonstrated success in effectively monitoring assay performance on the NeuMoDx System.

Table 13. Effectiveness of the Sample Process Control

Process Step Failure Tested	Sample Process Control Amplification Status	HCV 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 0.3 Log_{10} IU/mL of Control

Valid Results Rate

A retrospective analysis of data obtained during the performance evaluation of the NeuMoDx HCV Quant Assay on the NeuMoDx Systems was used for determination of the percentage of valid results. Valid test results will be called Positive or Negative; invalid test results may be reported as either Indeterminate (IND) or Unresolved (UNR) based on the amplification status of the target and the sample process control. An IND call is typically caused by instrument error leading to a failure of the target and/or internal process control to amplify. An UNR call is assigned to samples when both the target and the internal process control fail to amplify in the absence of a detected instrument failure. There were 1,962 individual NeuMoDx HCV Quant Assay results included in the retrospective analysis, which included data obtained from both serum and plasma specimens on both the NeuMoDx 288 and NeuMoDx 96 Systems. The UNR rate was determined to be 0.61% (12/1962) and the IND rate was determined to be 0.41% (8/1962); which meet the acceptance criteria of the analysis. Therefore, the valid result rate of the NeuMoDx HCV Quant Assay across clinical matrices and NeuMoDx Systems was concluded to be 99.0% with 95% CI (98.4-99.3).

Cross-contamination

The cross-contamination rate for NeuMoDx HCV Quant Assay was determined by testing three sets of HCV specimens featuring alternating high positive and negative specimens. In total, this involved testing 144 replicates of HCV-negative human specimen and 144 replicates of a high-titer HCV specimen at 8.2 Log_{10} IU/mL. All 144 replicates of the negative specimen were reported as negative, which demonstrates no cross-contamination occured during sample processing on the NeuMoDx System.

Specimen Matrix Equivalence

Testing was performed to demonstrate specimen matrix equivalency between whole blood collected in both ethylenediaminetetraacetic acid (EDTA) and acid citrate dextrose (ACD) collection tubes for the preparation of plasma. Additional testing was performed to determine equivalency between fresh and frozen plasma specimens (collected in the two tube types) as well as fresh and frozen serum specimens. Fresh specimens were kept at 4 °C until they were spiked with four levels of HCV and tested for equivalency. Next, the samples were frozen for a minimum of 24 hours at -20°C. Following this period of frozen storage, the specimens were thawed and re-tested. Results from fresh v. frozen serum and plasma as well as EDTA v. ACD plasma specimens were compared for equivalency by regression analysis. The data demonstrated excellent equivalency between EDTA and ACD plasma specimens, fresh and frozen plasma specimens, and fresh and frozen serum specimens.

Additional testing was performed to demonstrate equivalency of NeuMoDx HCV Quant Assay performance on primary specimens v. secondary specimens. Panels of HCV negative donor specimens spiked with HCV target (AccuPlex™ Recombinant HCV Control) and of HCV positive donor specimens were first processed from the primary specimen tubes. After primary tube processing, the remaining plasma or serum 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.

Clinical Method Comparison

Qualitative and quantitative performance of the NeuMoDx HCV Quant Assay were assessed against FDA/CE-approved comparator assays by testing undiluted clinical specimens from HCV infected patients. Testing was performed internally at NeuMoDx through a single-blinded study of de-identified, residual, clinical specimens obtained from six external reference laboratories. A total of 323 plasma specimens and 336 serum specimens were processed using NeuMoDx HCV Quant Assay in a (single) blinded manner across multiple NeuMoDx Molecular Systems. Of these samples, 35 plasma samples and 13 serum samples were processed on BOTH the NeuMoDx 288 and 96 Molecular Systems. Some of the samples that gave an INVALID result could not be processed again due to lack of availability of sufficient sample.

The processing and system errors obtained across the NeuMoDx Molecular Systems were minimal and met the criteria. A total of 4 indeterminate (IND) results were intially obtained for plasma samples and 4 IND results were obtained for serum samples, which resulted in an overall initial IND rate of 1% (95% CI 0.5% - 3%) for plasma, and 1% (95% CI 0.4%-3%) for serum. A total of 3 UNRESOLVED (UNR) results were initially obtained for plasma samples and 5 UNR for serum samples were obtained which yielded an overall rate of 1% (95% CI 0.2% - 3%) for plasma, and 1% (95% CI 0.6% - 4%) for serum.

Specimens yielding invalid results (IND/UNR) or a “Quantitation Error” were repeat tested when sufficient volume remained; a dilution step was performed on some samples in order to yield valid results. Of the 13 specimens that had sufficient volume to be repeated (diluted OR neat), a valid result was obtained.

Of the 321 valid results obtained for plasma specimens and 334 valid results obtained for serum samples, 206 plasma samples and 154 serum samples were reported POSITIVE by the NeuMoDx HCV Quant Assay with corresponding concentration values assigned by the reference tests. Deming Regression and Passing-Bablok Regression analyses were used to correlate between the concentration values of the NeuMoDx HCV Quant Assay and the values reported by the reference tests for both plasma and serum samples.

Equivalency plots were generated to represent the correlation between the NeuMoDx HCV Quant Assay concentrations and the reference test concentration values for all samples tested using the Deming Regression fit and Passing-Bablok fit and are presented in *Figure 7* and *Figure 8*. The quality of the Deming Regression fit is illustrated by a slope coefficient of 1.00 with a 95% CI (0.97, 1.03), and an intercept (bias) of -0.16 with a 95% CI (-0.37, 0.06), demonstrating that the concentration results obtained between the NeuMoDx HCV 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 1.02 with a 95% CI (0.99, 1.05), and an intercept (bias) of -0.28 with a 95% CI (-0.43, -0.14), demonstrating that the concentration results obtained between the NeuMoDx HCV Quant Assay and Reference tests are highly correlated and with acceptable bias as shown in *Table 14*.

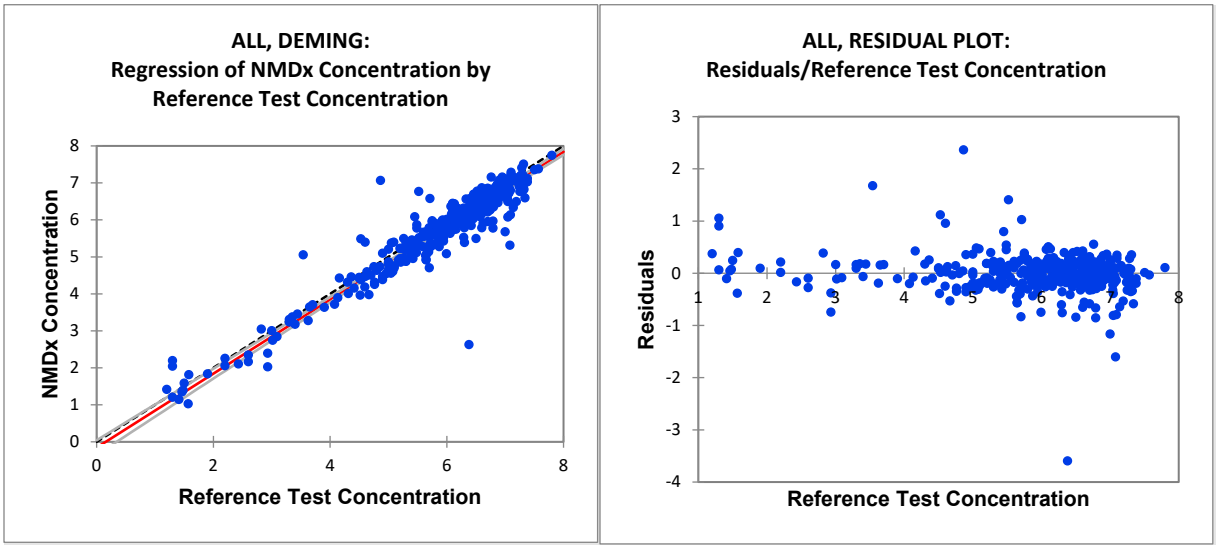

Figure 7: Equivalency (left) and Residual (right) Plots – Cumulative Analysis (across both NeuMoDx Systems) of NeuMoDx HCV Quant Assay Results Compared to Reference Test Results for ALL samples based on Deming Regression Analysis.

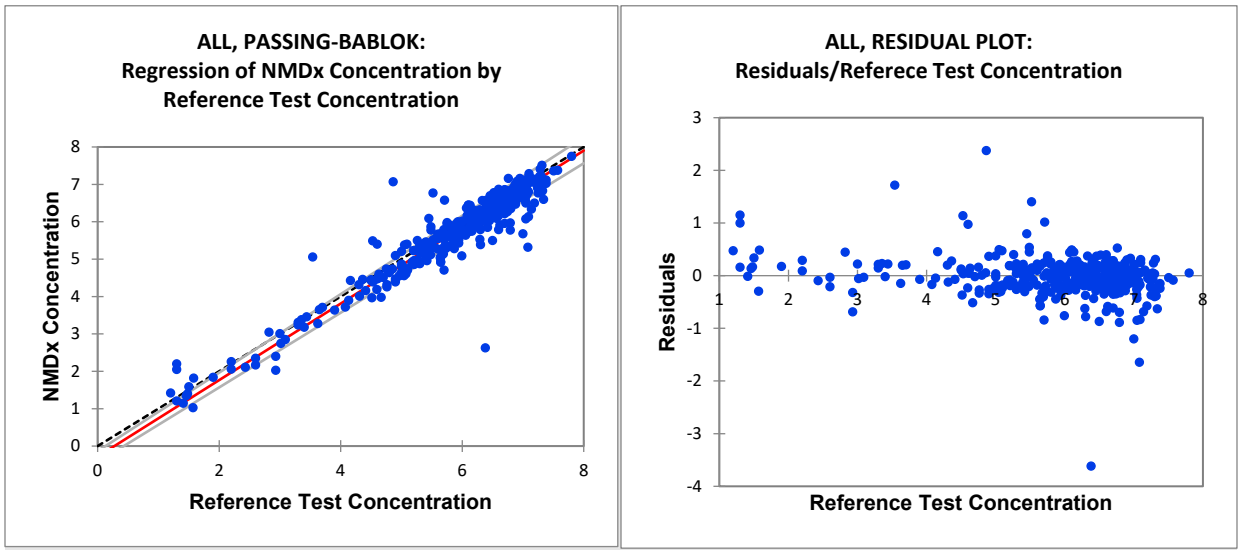

Figure 8: Equivalency (left) and Residual (right) Plots – Cumulative Analysis (across both NeuMoDx Systems) of NeuMoDx HCV Quant Assay Results Compared to Reference Test Results for ALL samples based on Passing-Bablok Regression Analysis.

Table 14. Summary of Deming and Passing-Bablok Linear Regression Analysis

	Deming Analysis		Passing-Bablok Analysis	
	Intercept	Slope Coefficient	Intercept	Slope Coefficient
CUMULATIVE (All Plasma + Serum)	- 0.16 95%CI (-0.37,0.06)	1.00 95%CI (0.97,1.03)	- 0.28 95%CI (-0.43,-0.14)	1.02 95%CI (0.99,1.05)

Of the 655 valid results obtained for plasma and serum specimens using the NeuMoDx HCV Quant Assay, 361 were reported positive by the reference tests for HCV and 294 were reported negative. The Sensitivity and Specificity of the NeuMoDx HCV Quant Assay were calculated using the data from all valid clinical samples as compared to the reference test, which is compiled and presented in *Table 15*. Of the 361 positive samples tested, 360 were also reported positive by the NeuMoDx HCV Quant Assay, demonstrating 99.7% sensitivity with 95% CI (98.2% - 100%). Of the 294 negative samples tested, 271 were also reported negative by the NeuMoDx HCV Quant Assay, demonstrating 92.2% specificity with 95% CI (88.3% - 94.9%).

Equivalency of the NeuMoDx HCV Quant Assay was demonstrated through highly correlated results of assay performance among the NeuMoDx 288 Molecular System, NeuMoDx 96 Molecular System, and reference test for both plasma and serum specimens.

Table 15. Qualitative Method Comparison Results for NeuMoDx HCV Quant Assay compared to Reference Tests - Plasma and Serum

	Reference Assay (POS)	Reference Assay (NEG)	TOTAL
NeuMoDx HCV Quant Assay (POS)	360	23	383
NeuMoDx HCV Quant Assay (NEG)	1	271	272
TOTAL	361	294	655
SENSITIVITY = 99.7% 95% CI (98.2% - 100%) ***SPECIFICITY = 92.2%** 95% CI (88.3% - 94.9%)			

***NOTE:** *The LLoQ for NeuMoDx HCV Quant Assay is 0.9 Log10 IU/mL, which is lower than the comparator assay used as the reference test. A subsequent analysis was performed excluding 9 samples where HCV was detected by the NeuMoDx, but reported as negative by the comparator assay. With the exclusion of these 9 samples, the specificity of the NeuMoDx HCV Quant Assay was re-calculated to be 95.1% with a 95% CI (91.7 – 97.2).*

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, HCV-negative plasma and serum samples spiked with four known levels of Accuplex HCV Control material, traceable to the 5th WHO International Standard for HCV RNA for nucleic acid tests. These individual plasma and serum specimens were processed using the 550 µL and 200 µL specimen volume workflows for a total of 324 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 1.003 and 1.000 with intercepts of -0.082 and -0.085, respectively in plasma and 0.974 and 0.984 with intercepts of 0.086 and 0.037, respectively in serum, demonstrating excellent concordance of HCV quantifications between the two processing volume worlflows. 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 1.0432 and a correlation coefficient of 0.994 (plasma) and of 1.0007 and 0.993 (serum), further supporting excellent performance using the 200 µL specimen volume workflow for the NeuMoDx HCV Quant Assay. Results of these studies are summarized below in *Figure 9 and Figure 10.*

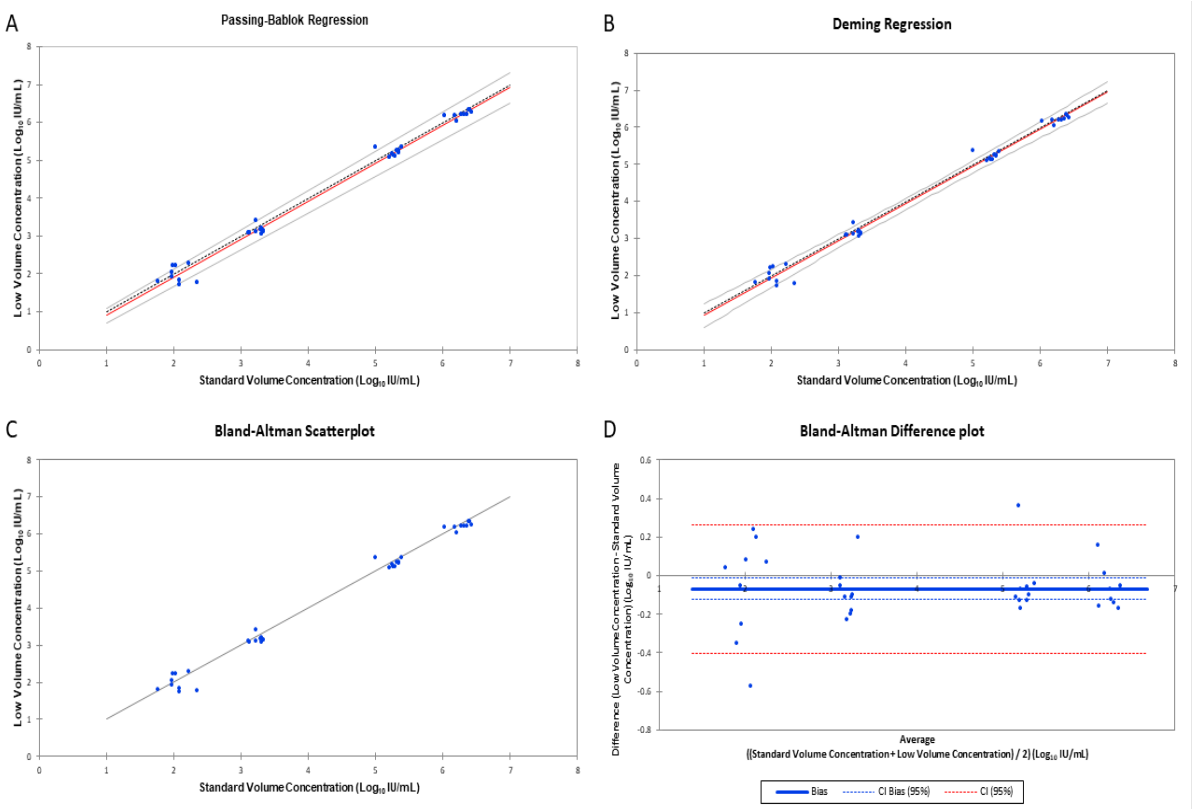

Figure 9: Equivalency Plot Comparisons of 200 µL Specimen Volume Workflow Reported Concentrations to 550 µL Specimen Volume Workflow Reported Concentrations. A) Passing-Bablok Regression. B) Deming Regression. C) Bland-Altman Scatterplot D) Bland-Altman Difference Plot – Plasma Specimens

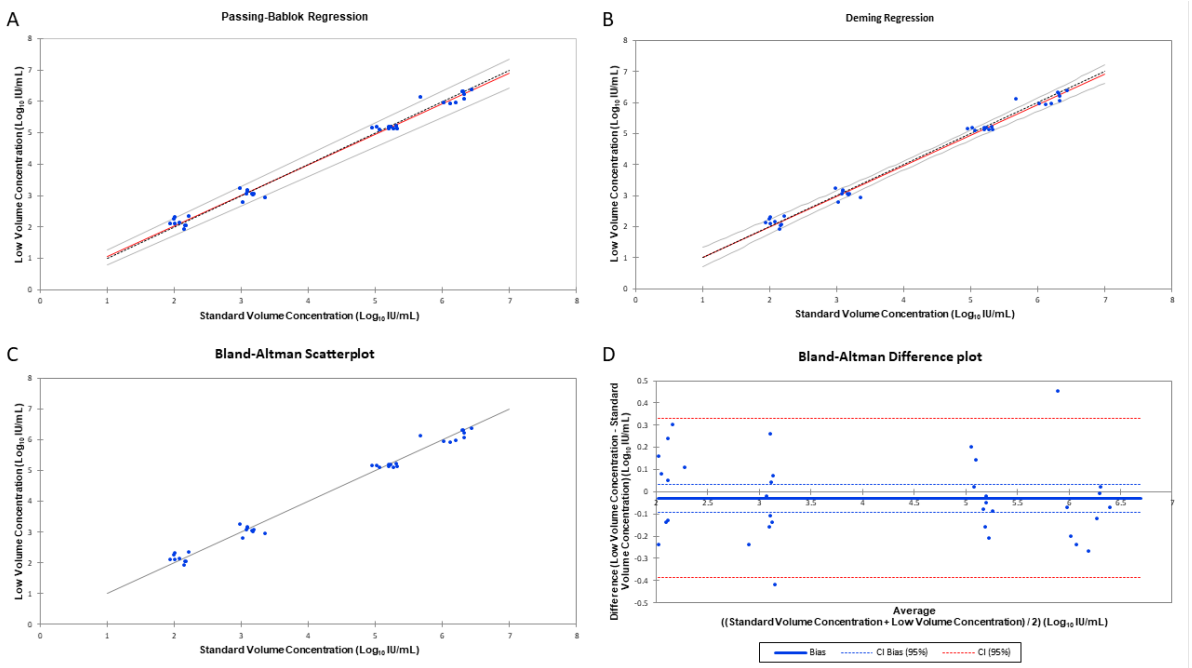

Figure 10: Equivalency Plot Comparisons of 200 µL Specimen Volume Workflow Reported Concentrations to 550 µL Specimen Volume Workflow Reported Concentrations. A) Passing-Bablok Regression. B) Deming Regression. C) Bland-Altman Scatterplot

D) Bland-Altman Difference Plot – Serum Specimens

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TRADEMARKS

SYMBOL KEY

℞ only	Prescription use only		Temperature limit
	Manufacturer		Do not re-use
IVD	*In vitro* diagnostic medical device	Σ	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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