REF 300500 NeuMoDx™ HIV-1 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 HIV-1 Quant Assay, performed on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System (NeuMoDx System(s)), is an automated, quantitative and qualitative *in vitro* diagnostic nucleic acid amplification test designed for quantitation and detection of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma.

The NeuMoDx HIV-1 Quant Assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis for use as an aid in clinical management of HIV-1 infected patients and monitoring the effects of antiretroviral treatment, as measured by changes in plasma HIV-1 RNA levels. The assay can quantitate HIV-1 RNA over the range of 34.2 to 5.0 x 10^7 IU /mL (1.5-7.7 $\log_{10}$ IU/mL). The NeuMoDx HIV-1 Quant Assay is validated for quantification of RNA from HIV-1 group M (subtypes A, B, C, D, F, G, H, K, CRF01_AE, CRF02_AG) N, O, and P.

The NeuMoDx HIV-1 Quant Assay is intended as an aid in the diagnosis of HIV-1 infection, including acute or primary infection. Presence of HIV-1 RNA in the plasma of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The NeuMoDx HIV-1 Quant Assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays and as a confirmation of HIV-1 infection.

The NeuMoDx HIV-1 Quant Assay is not intended to be used as a donor screening test for HIV-1 for the presence of HIV-1 in blood or blood products.

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. To prepare for testing, plasma 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 to begin processing. For each specimen, a 600 µL aliquot of the plasma 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 reverse transcription polymerase chain reaction (RT-PCR) and, if present, amplify and detect the products of amplification (sections of the HIV-1 genome in conserved regions). The NeuMoDx HIV-1 Quant Assay includes an RNA Sample Process Control (SPC2) to help monitor for the presence of potential inhibitory substances and NeuMoDx System or reagent failures that may be encountered during the extraction and amplification process.

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS) and is divided into two major types, the more common and pathogenic of which is HIV type 1 (HIV-1). HIV-1 can be transmitted through sexual contact, exposure to infected blood or blood products, or from an infected mother to the fetus.[1-4] Acute HIV-1 infection, characterized by flu-like symptoms, develops 3 to 5 weeks after initial infection and is associated with high levels of viremia. HIV-1 specific immune response is detectable within 4 to 6 weeks of the onset of symptoms.[5-9]

Upon seroconversion, most patients enter an asymptomatic phase that can last for years. Quantitative measurement of HIV-1 RNA levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV-1 infection and has been shown to be an essential parameter in prognosis and management of HIV-1 infected individuals.[10-11] Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma HIV-1 RNA levels (viral load), CD4+ T-cell count, and the patient's clinical condition.[12-17] The goal of antiretroviral therapy is to suppress HIV-1 replication to below detectable levels of currently available viral load tests. Virus levels in the peripheral blood can be quantitated by measurement of the HIV p24 antigen in serum, by quantitative culture of HIV from plasma, or by direct measurement of viral RNA in plasma using nucleic acid amplification or signal amplification technologies.[9-11] Molecular techniques such as reverse transcription mediated polymerase chain reaction have been widely used to amplify nucleic acids.[11] The NeuMoDx HIV-1 Quant Assay uses RT-PCR technology with homogenous real-time fluorescence detection. The assay includes dual-target amplification and detection, targeting two independent regions of the HIV-1 genome. In addition, degenerate assay design allows detection of diverse group M subtypes (A, B, C, D, F, G, H, K), including circulating recombinant forms, and group N, O, and P isolates. The assay results are reported in International Units per mL (IU/mL).

PRINCIPLES OF THE PROCEDURE

The NeuMoDx HIV-1 Quant Assay combines automated RNA extraction and amplification/detection by real-time RT-PCR. Whole blood specimens are collected in EDTA, ACD, or PPT tubes for the preparation of plasma. The primary (fractionated) blood specimen or a plasma 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 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 HIV-1 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 RT-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) using 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 HIV-1 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 result (POSITIVE/NEGATIVE/INDETERMINATE/UNRESOLVED). 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	Tests per unit	Tests per package
300500	**NeuMoDx HIV-1 Quant Test Strip** *Dried RT-PCR reagents containing HIV-1 and SPC2 specific TaqMan probe and primers*	16	96

Additional Materials Required (Available Separately)

REF	Contents
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
800304	**NeuMoDx HIV-1 Calibrators** *Single use sets of HIV-1 High and Low Calibrators to establish validity of standard curve*
900301	**NeuMoDx HIV-1 External Controls** *Single use sets of HIV-1 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 & PRECAUTIONS

- The NeuMoDx HIV-1 Quant Test Strip is for *in vitro* diagnostic use with NeuMoDx Molecular 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 HIV-1 Calibrators [REF 800304]) must exist before test results can be generated for clinical samples.
- External controls (from the NeuMoDx HIV-1 External Controls [REF 900301]) must be processed every 24 hours hours throughout testing with the NeuMoDx HIV-1 Quant Assay.
- Minimum specimen volume of secondary aliquots is dependent on the tube size/specimen tube carrier 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 ribonuclease (RNase) contamination of all reagents and consumables. The use of sterile RNase-free, disposable transferring pipettes is recommended when 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 HIV-1 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 HIV-1 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*[18] and in CLSI Document M29-A4.[19]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

PRODUCT STORAGE, HANDLING & STABILITY

- NeuMoDx HIV-1 Quant Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 15 - 23 °C.
- NeuMoDx HIV-1 Quant Test Strips are shipped in an insulated container that contains gel coolant packets.
- 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 HIV-1 Quant Test Strip may remain onboard the NeuMoDx System for seven (7) 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.
- Although non-infectious, discard NeuMoDx calibrators and external controls after use in laboratory biohazard waste to reduce risk of contamination by the target nucleic acid contained.

SPECIMEN COLLECTION, TRANSPORT & 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. Specimens may be tested in primary collection tubes or secondary specimen tubes. Recommended for primary tube testing: BD Vacutainer® Plus Plastic K_2EDTA Tube (BD #368589) or BD Vacutainer PPT™ Plasma Preparation Tube (BD #362799).
5. Prepared plasma 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 as secondary plasma aliquots.
6. Prepared plasma specimens should be stored at 2 to 8 °C for no longer than 7 days prior to testing and a maximum of 8 hours at room temperature.

7. Prepared specimens may be stored at ≤ -20°C for up to 8 weeks for plasma before processing.
    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 8 hours.
    c. Plasma samples should not be subjected to more than 4 freeze/thaw cycles prior to use
8. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.
9. Label specimens clearly and indicate specimens are for HIV-1 testing.
10. Proceed to *Test Preparation* section.

The overall process for implementation of the NeuMoDx HIV-1 assay is summarized below in *Figure 1*.

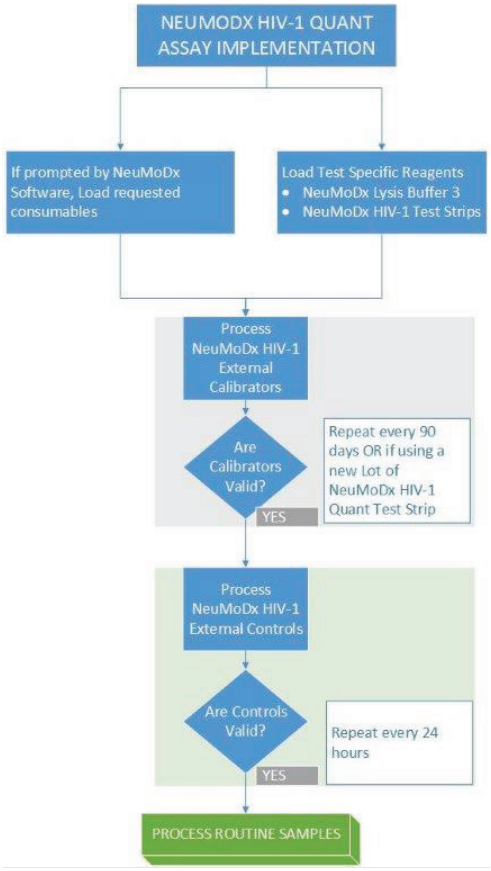

Figure 1: NeuMoDx HIV-1 Quant Assay Implementation Workflow

INSTRUCTIONS FOR USE

Test Preparation

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

    - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 750 µL
    - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 1200 µL
    - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 700 µL

NeuMoDx System Operation

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

1. Populate one or more NeuMoDx System Test Strip carrier(s) with NeuMoDx HIV-1 Quant Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
2. 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.
3. 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.
4. If prompted by the NeuMoDx System software, process NeuMoDx HIV-1 Calibrators [REF 800304] and/or NeuMoDx HIV-1 External Controls [REF 900301]. Further information regarding calibrators and controls can be found in the *Results Processing* section.
5. Load the specimen/calibrator/control tube(s) into a Specimen Tube Carrier and ensure caps are removed from all tubes.
6. 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 HIV-1 Quant Test Strip can only be used on NeuMoDx Molecular Systems.
2. The performance of the NeuMoDx HIV-1 Quant Test Strip has been established for plasma specimens prepared from whole blood collected with EDTA/ACD as the anticoagulant. Use of the NeuMoDx HIV-1 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 HIV-1 Quant Test Strip has been established for primary tube testing using BD Vacutainer Plus Plastic K_2EDTA Tubes and BD Vacutainer PPT™ Plasma Preparation Tube.
4. The NeuMoDx HIV-1 Quant Assay must not be used with samples from heparinized humans.
5. Because detection of HIV-1 is dependent on the number of viral particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
6. NeuMoDx HIV-1 Calibrators and NeuMoDx HIV-1 External Controls must be processed as recommended in the package inserts when prompted by NeuMoDx System software before processing routine clinical samples.
7. Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube mix-up. 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 HIV-1 Quant Assay.
8. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
9. If both the HIV-1 target and the SPC2 target do not amplify, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
10. If the NeuMoDx HIV-1 Quant Assay result is Positive, but the quantitation value is beyond the limits of quantitation, the NeuMoDx System will report whether the detected HIV-1 was below Lower Limit of Quantitation (LLoQ) or above Upper Limit of Quantitation (ULoQ).
11. In the event the detected HIV-1 was below LLoQ, the NeuMoDx HIV-1 Quant Assay may be repeated (if desired) with another aliquot of the specimen.
12. In the event the detected HIV-1 is above ULoQ, the NeuMoDx HIV-1 Quant Assay may be repeated with a diluted aliquot of the original specimen. A 1:100 or 1:1000 dilution in HIV-1 negative plasma or Basematrix 53 Diluent (Basematrix) (SeraCare, Milford, MA) is recommended. The concentration of the original specimen can be calculated as follows:

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

13. 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.
14. A positive result does not necessarily indicate the presence of viable HIV-1. However, a positive result is presumptive for the presence of HIV-1 RNA.
15. Deletions or mutations in the conserved regions targeted by the NeuMoDx HIV-1 Quant Assay may affect detection and could lead to an erroneous result.
16. Results from NeuMoDx HIV-1 Quant Assay should be used as an adjunct to clinical observations and other information available to the physician.
17. 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 HIV-1 Quant Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx HIV-1 Assay Definition File (HIV-1 ADF). A NeuMoDx HIV-1 Quant Assay result may be reported as Negative, Positive with a reported HIV-1 concentration, Positive above ULoQ, Positive below LLoQ, Indeterminate, or Unresolved, 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 HIV-1 Quant Assay Decision Algorithm

RESULT*	HIV-1 Target(s)	Sample Process Control (SPC2)
Positive with Reported Concentration	Amplified, 1.5 ≤ [HIV-1] ≤ 7.7 $\log_{10}$ IU/mL	Amplified or Not Amplified
Positive, above ULoQ	Amplified, [HIV-1] > 7.7 $\log_{10}$ IU/mL	Amplified or Not Amplified
Positive, below LLoQ	Amplified, [HIV-1] < 1.5 $\log_{10}$ IU/mL	Amplified or Not Amplified
Negative	Not Amplified	Amplified
Indeterminate	Not Amplified, System Error Detected	
Unresolved	Not Amplified, No System Error Detected	

*The quantification range of the NeuMoDx HIV-1 Quant Assay is 1.5 to 7.7 $\log_{10}$ IU/mL. A POSTIVE result indicates HIV-1 RNA is detected and aids in the diagnosis of HIV-1 infection. A NEGATIVE result indicates either the absence of HIV-1 RNA or the viral load is below the limit of detection. False-negative or falsely low viral load results may be caused by improper specimen collection or storage. The results must be interpreted within the context of relevant clinical and laboratory findings.

Test Calculation

1. For samples within the quantitation range of the NeuMoDx HIV-1 Quant Assay, the concentration of HIV-1 RNA in the samples is calculated using the stored standard curve in conjunction with the calibration coefficient.
    a. A calibration coefficient is calculated based on the results of the NeuMoDx HIV-1 Calibrators processed to establish validity of the standard curve for a given lot of the NeuMoDx HIV-1 Quant Test Strip on a specific NeuMoDx System.
    b. The calibration coefficient is incorporated into the final determination of the concentration of HIV-1 RNA.
2. NeuMoDx HIV-1 Quant Assay results are reported in $\log_{10}$ IU/mL. The conversion factor for NeuMoDx HIV-1 Quant Assay is 0.75 copy/IU.
3. The resulting quantitation of unknown samples is traceable to a calibrated reference material obtained from the National Institute for Biological Standards and Control.

Test Calibration

A valid calibration based on the standard curve is required to quantitate HIV-1 RNA in the specimens. To generate valid results, a test calibration must be completed using the calibrators provided by NeuMoDx Molecular, Inc.

Calibrators

1. NeuMoDx HIV-1 Calibrators [REF 800304] contain non-infectious, encapsulated HIV-1 target prepared in Basematrix.
2. A set of the HIV-1 calibrators need to be processed with each new lot of NeuMoDx HIV-1 Quant Test Strips, if a new HIV-1 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.
3. 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.
4. 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 HIV-1 concentration.
5. 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.

6. 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. NeuMoDx HIV-1 External Controls [REF 900301] contain positive controls of non-infectious, encapsulated HIV-1 target prepared in Basematrix and negative controls of Basematrix only.
2. Positive and negative external controls need to be processed every 24 hours throughout testing with the NeuMoDx HIV-1 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.
3. Validity of external controls will be assessed by the NeuMoDx System based on the expected result. The positive control should provide an HIV-1 Positive result and the negative control should provide an HIV-1 Negative result.
4. 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, repeat the NeuMoDx HIV-1 External Controls with fresh vials of the control(s) failing the validity test.
    d) If positive NeuMoDx HIV-1 external control continues to report a Negative result, contact NeuMoDx technical service.
    e) If negative NeuMoDx HIV-1 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 to SPC2 are also included in each NeuMoDx HIV-1 Quant Test Strip, enabling detection of SPC2 with the target HIV-1 RNA (if present) via multiplex 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 HIV-1 Quant Assay performed on the NeuMoDx System fails to produce a valid result, it will be reported as either Indeterminate (IND) 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.

A UNR result will be reported if no valid amplification of HIV-1 RNA or SPC2 is detected, which indicates possible reagent failure or the presence of inhibitors. If a 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.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity – Limit of Detection

The analytical sensitivity of the NeuMoDx HIV-1 Quant Assay was characterized by testing a dilution series traceable to the WHO 3rd HIV-1 International Standard in screened HIV-1 RNA negative EDTA plasma to determine the limit of detection (LoD) on the NeuMoDx Systems. The LoD is defined as the lowest target level detected at a rate of ≥ 95% as determined by probit analysis. The study was performed over three (3) days using multiple systems, operators, runs, and lots of NeuMoDx HIV-1 Quant Assay reagents. Each system processed 12 replicates at each dilution level per day. Detection rates are depicted in *Table 2*.

Table 2: Positive Detection Rates for LoD Determination of the NeuMoDx HIV-1 Quant Assay

Target Concentration (IU/mL)	Target Concentration (log_{10} IU/mL)	Number of Valid Tests	Number of Positives	Detection Rate (%)
60	1.78	72	71	98.6%
45	1.65	72	71	98.6%
35	1.54	72	68	94.4%
15	1.18	72	54	75.0%
0	-	72	0	0%

Through probit analysis, the LoD of the NeuMoDx HIV-1 Quant Assay in plasma across all genotypes was determined to be **34.2 IU/mL (1.5 log_{10} IU/mL)** with 95% confidence interval (CI) of 27.8 to 47.7 IU/mL (1.4-1.7 log_{10} IU/mL) as tested on the NeuMoDx 288 Molecular System [*Figure 2*].

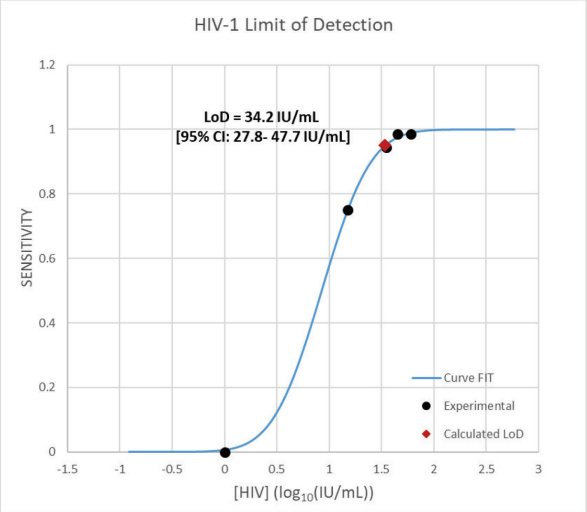

Figure 2: Probit Analysis of the NeuMoDx HIV-1 Quant Assay Limit of Detection

Analytical Sensitivity – Lower Limit of Quantitation

The lower limit of quantitation (LLoQ) is defined as the lowest target level at which > 95% detection is achieved and total analytical error ≤ 1. To determine the LLoQ, the total analytical error (TAE) was calculated for each of the HIV-1 target levels as part of LoD calculation. TAE is defined as follows:

TAE = bias + 2*SD (Westgard Statistic)

where

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

Compiled results for the four (4) levels of HIV-1 plasma specimens used in the LLoQ study using subtype B are shown in *Table 3*. Because the calculated TAE was ≤ 1 at HIV-1 levels below the LoD, the NeuMoDx HIV-1 Quant Assay demonstrated a lower limit of quantitation equivalent to the limit of detection: **34.2 IU/mL** (95% CI 27.8-47.7 IU/mL) or **1.5 $\log_{10}$ IU/mL** (95% CI 1.4-1.7 $\log_{10}$ IU/mL).

Table 3: NeuMoDx HIV-1 Quant Assay LLoQ, with Bias and TAE

Target Conc. (IU/mL)	Target Conc. ($\log_{10}$ IU/mL)	Average Conc. ($\log_{10}$ IU/mL)	Detection (%)	SD	Bias	TAE
60	1.78	1.76	99	0.28	0.02	0.59
45	1.65	1.82	99	0.30	0.17	0.78
35	1.54	1.69	94	0.39	0.15	0.93
15	1.18	1.52	75	0.54	0.34	1.44

Analytical Sensitivity – Linearity and Determination of Upper Limit of Quantitation

Linearity and the upper limit of quantitation (ULoQ) of the NeuMoDx HIV-1 Quant Assay were established by preparing a dilution series of HIV-1 sourced from The External Quality Assurance Program Oversight Laboratory (Duke University, NC, USA), AccuPlex™ Recombinant HIV/HCV Control (Seracare, MA, USA) and HIV-1 RNA Working Reagent 2 for NAT Assays (NIBSC). A nine-member panel was prepared in pooled HIV-1 RNA negative EDTA plasma to span a concentration range of 7.70-1.70 $\log_{10}$ IU/mL. The NeuMoDx HIV-1 Quant Assay demonstrated the ability to quantify HIV-1 across the 6 $\log_{10}$ linear range with an accuracy of ± 0.33 $\log_{10}$ IU/mL based on the standard error as calculated by the 95% confidence interval. No significant benefit was gained using 2nd or 3rd order regression fits. The ULoQ was determined using the data from this study to be **7.7 $\log_{10}$ IU/mL**. The HIV-1 assay concentrations reported by the NeuMoDx System compared to the expected values are presented in *Figure 3.*

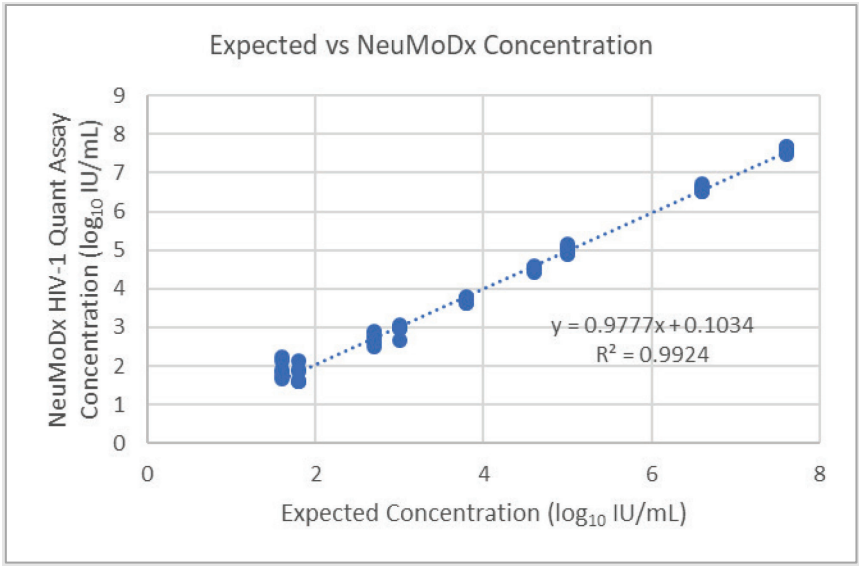

Figure 3: Linear range of the NeuMoDx HIV-1 Quant Assay

Analytical Sensitivity – Linearity Across Genotypes

The linearity of the NeuMoDx HIV-1 Quant Assay across HIV-1 groups M (subtypes A, B, C, D, F, G, H, K, CRF01_AE, CRF02_AG), N, O, and P was characterized by testing at least five (5) different concentrations of each group/subtype of HIV-1 prepared in pooled HIV-1 RNA negative EDTA plasma. The levels of HIV-1 target tested in this study were dependent on the concentration of the source specimen, and thus differed between groups/subtype. The study was performed with each group/subtype using six (6) replicates at each level. Linearity was demonstrated across the ranges tested and is presented in *Table 4* and *Figure 4*.

Table 4: Linearity of the NeuMoDx HIV-1 Quant Assay Across Groups M, N, O, and P

Group	Subtype	**Linearity Equation** *y = NeuMoDx HIV-1 Quant Assay Quantitation (log_{10} IU/mL)* *x = Expected Quantitation (log_{10} IU/mL)*	R^2
M	A	y = 1.0217x - 0.008	0.9953
	B	y = 0.9715x + 0.1442	0.9933
	C	y = 1.0055x + 0.0658	0.9879
	D	y = 1.0203x - 0.3554	0.9941
	F	y = 0.9872x + 0.4278	0.9955
	G	y = 1.0282x + 0.2223	0.9970
	CRF01_AE	y = 1.0163x - 0.0053	0.9824
	CRF02_AG	y = 0.99x - 0.0783	0.9989
	H	y = 0.9803x + 0.4187	0.9730
	K	y = 1.0441x - 0.0223	0.9684
N		y = 0.996x + 0.2117	0.9876
O		y = 1.0043x + 0.6167	0.9942
P		y = 0.9927x + 0.1903	0.9974

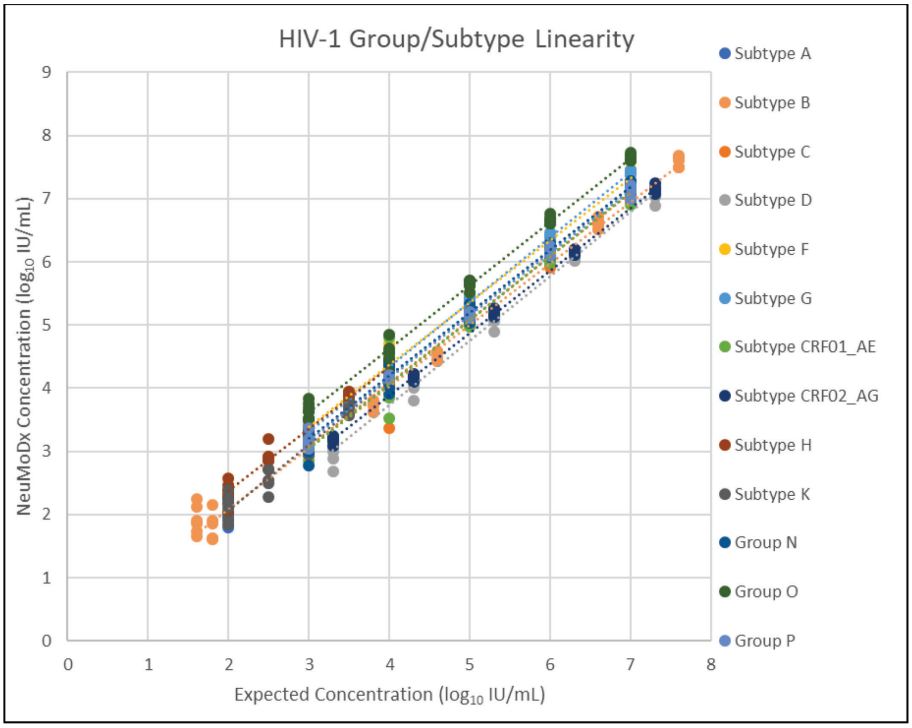

Figure 4: Linearity of the NeuMoDx HIV-1 Quant Assay across Subtypes

Analytical Specificity – Potentially Interfering Microbial Contaminants

The analytical specificity of the NeuMoDx HIV-1 Quant Assay was evaluated by testing a panel of microorganisms (*Table 5*) prepared in HIV-1 RNA negative EDTA plasma at high concentrations for cross-reactivity. Potential interference was assessed using the same panel of microorganisms prepared in EDTA plasma and spiked with HIV-1 at 2.02 $\log_{10}$ IU/mL. No cross-reactivity was observed, with all HIV-1 negative microbial samples yielding negative results. All HIV-1 positive microbial samples gave positive results, and no significant interference was observed in these samples as evidenced by minimal deviation in reported HIV-1 quantitation from control specimens containing no potentially interfering microorganisms. Further potential cross-reactivity was assessed by nucleotide sequence comparison of the NeuMoDx HIV Quant Assay target sequences with the complete genomes of 26 additional pathogens (*Table 6*) using the Basic Local Alignment Search Tool (BLASTn) made available by the National Center for Biotechnology Information (NCBI). The sequence comparison analysis showed no analogy between targeted sequences and the genomes examined.

Table 5: Pathogens Tested for Analytical Specificity

Potentially Interfering Microorganism
Hepatitis A Virus
Hepatitis B Virus
Hepatitis C Virus
Human T- cell leukemia virus type 1 (HTLV-1)
Human T- cell leukemia virus type 2 (HTLV-2)
Human Immunodeficiency virus type 2 (HIV-2)
Simian immunodeficiency virus (SIV)
Epstein-Barr Virus

Table 6: Microorganisms Included in BLASTn Sequence Alignment Analysis

Microorganism	Accession Number(s)	Microorganism	Accession Number(s)
Adenovirus Type 12	X73487.1	Human herpesvirus 5	GQ221974.1 KR534211.1 GQ221975.1 NC_006273.2
BK polyomavirus	AB369101.1 NC_001538.1 AB369092.1	Human herpesvirus 7	AF037218.1 NC_001716.2
Chlamydia trachomatis	CP018052.1 CP017731.1	Human herpesvirus 8	NC_009333.1
Cutibacterium acnes	NZ_CP006032.1	Human papillomavirus type 18	NC_001357.1 MF288723.1
Dengue virus	KR919821.1 KR052012.1	Human papillomavirus type 16	KY549222.1 KY549321.1
Herpex Simplex virus type 2	Z86099.2	Human parvovirus B19	KX752821.1 MH201456.1
Human Adenovirus 2	J01917.1 AC_000007.1	Influenza A (all segments)	MN253846.1 MH797924.1 MH842686.1 MN037420.1
Human Adenovirus 5	KX868466.2 AC_000008.1 AY601635.1	JC virus	J02226.1 AB081030.1
Human Adenovirus C	AY339865.1	*Neisseria gonorrhoeae*	CP034022.1 CP041586.1
Human betaherpesvirus 6A	NC_001664.4 X83413.2	*Propionibacterium acnes C1*	CP003877.1
Human herpesvirus 1	X14112.1 JQ780693.1	*Staphylococcus aureus*	AP017922.1
Human herpesvirus 2	LT797626.1 JN561323.2	*Staphylococcus saprophyticus*	AP008934.1
Human herpesvirus 3	DQ479962.1 KC847290.1	West Nile virus	M12294.2 MF797870.1

Analytical Specificity – Potentially Interfering Endogenous and Exogenous Substances

The NeuMoDx HIV-1 Quant Assay was evaluated for susceptibility to interference by drugs commonly prescribed to HIV-1 infected individuals, elevated levels of endogenous substances, and by the presence of autoimmune diseases. Screened HIV-1 RNA negative EDTA plasma was spiked with 3 $\log_{10}$ IU/mL HIV-1 and with albumin (120 mg/mL), bilirubin (0.03 mg/mL), hemoglobin (3.5 mg/mL), triglycerides (5.3 mg/mL), and drug compounds (*Table 7*) at three times the C_{max}. Disease state plasma for systemic lupus erythematosus (SLE), antinuclear antibody (ANA), and rheumatoid arthritis (RA) were likewise screened negative and spiked with 3 $\log_{10}$ IU/mL HIV-1 for testing. No significant interference was observed. Results of the study are summarized in *Table 8*.

Table 7: Drug Compounds Tested for Interference

Drug Classification	Drug Name
Immune Modulator	Interferon alfa-2a, Interferon alfa-2b, Ribavirin
CCR5 Antagonist	Maraviroc
Pharmacokinetic Enhancer	Cobicistat
Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)	Doravirine, Efavirenz, Nevirapine, Rilpivirine
Protease Inhibitor (PI)	Darunavir, Amprenavir, Ritonavir, Saquinavir, Simeprevir
Nucleoside Reverse Transcriptase Inhibitor (NRTI) or DNA Polymerase Inhibitor	Cidofovir, Lamivudine, Ganciclovir, Tenofovir disoproxil, Zidovudine, Valganciclovir, Abacavir sulfate, Emtricitabine, Entecavir, Foscarnet, Sofosbuvir
Integrase Inhibitor	Raltegravir, Dolutegravir
Fusion Inhibitor	Enfuvirtide
Opportunistic Infection Treatment	Azithromycin, Clarithromycin, Fluconazole, Sulfamethoxazole, Trimethoprim

Table 8: Interference Testing Summary - Exogenous and Endogenous Agents

Endogenous	Average [HIV-1] ($\log_{10}$ IU/mL)	Bias ($\log_{10}$ IU/mL)
Albumin	3.03	-0.11
Bilirubin	3.04	-0.09
Hemoglobin	3.04	-0.09
Triglycerides	3.14	0.01
Exogenous (Drugs)	**Average [HIV-1] ($\log_{10}$ IU/mL)**	**Bias ($\log_{10}$ IU/mL)**
Pool 1: Interferon alfa-2a, Interferon alfa-2b, Ribavirin, Maraviroc, Cobicistat	3.06	-0.07
Pool 2: Raltegravir, Dolutegravir, Efavirenz, Nevirapine, Rilpivirine	3.04	-0.09
Pool 3: Doravirine, Darunavir, Amprenavir, Ritonavir, Saquinavir	3.11	-0.02
Pool 4: Simeprevir, Enfuvirtide, Abacavir sulfate, Emtricitabine, Entecavir, Foscarnet	3.12	-0.01
Pool 5: Cidofovir, Lamivudine, Ganciclovir, Tenofovir disoproxil, Zidovudine, Valganciclovir	3.14	0.01
Pool 6: Sofosbuvir, Azithromycin, Clarithromycin, Fluconazole, Sulfamethoxazole, Trimethoprim	3.13	0
Disease State	**Average [HIV-1] ($\log_{10}$ IU/mL)**	**Bias ($\log_{10}$ IU/mL)**
Systemic Lupus Erythematosus (SLE)	3.00	-0.13
Antinuclear Antibody (ANA)	3.10	-0.03
Rheumatoid Arthritis (RA)	3.25	0.12

Precision

Precision of the NeuMoDx HIV-1 Quant Assay was determined by testing a four-member panel of HIV-1 samples prepared in HIV-1 negative plasma (incorporating both HIV-1 Subtype B and Group O from EQAPOL, Duke University) on three (3) NeuMoDx Systems across six (6) days. A total of 12 runs were made on each system for each sample level, resulting in 216 replicates per level over the span of testing. The within-run, within-day and within-System precisions were characterized, and the overall standard deviation was determined to be ≤ 0.15 $\log_{10}$ IU/mL. No significant difference

was found in performance across systems, days, or runs, as shown in *Table 9*. Precision between operators was not characterized as the operator plays no significant role in the processing of samples using the NeuMoDx System.

Table 9: Within Lab Precision – NeuMoDx HIV-1 Quant Assay on NeuMoDx Systems

	Target Conc. (log_{10} IU/mL)	Avg Conc. (log_{10} IU/mL)	Within System SD	Within Day SD	Within Run SD	Within Lab (Overall) SD
Subtype B	5.7	5.62	0.09	0.09	0.09	0.10
	3.7	3.62	0.10	0.10	0.10	0.13
Group O	4.7	4.65	0.09	0.09	0.09	0.12
	2.7	2.66	0.13	0.13	0.12	0.15

Lot-to-Lot Variation

Lot-to-lot reproducibility of the NeuMoDx HIV-1 Quant Assay was verified by retrospective analysis of quality test data for three (3) distinct lots of critical reagents. These data were generated through functional testing of the reagents on a three-member panel of HIV target (AccuPlex Recombinant HIV/HCV Control) in HIV-1 RNA negative plasma, along with negative plasma samples. A total of 18 positive and 14 negative replicates were processed per lot of NeuMoDx HIV-1 Quant Test Strip. The variation within and across lots was analyzed and presented in *Table 10.* Overall absolute bias did not exceed 0.14 log_{10} IU/mL and overall standard deviation fell below 0.25 log_{10} IU/mL. No significant difference was found in performance across lots, as quantitation of all panel members was within tolerance specification.

Table 10: Lot-to-Lot Reproducibility – NeuMoDx HIV-1 Quant Assay

Target Conc. (log_{10} IU/mL)	Mean Conc. Overall (log_{10} IU/mL)	Number of Valid Tests	|Bias| (log_{10} IU/mL)	Between Lot SD	Within Lot SD	Overall SD
5.00	4.96	18	0.04	0.08	0.08	0.12
3.00	2.86	17	0.14	0.12	0.18	0.22
2.00	1.92	18	0.08	0.17	0.14	0.22

Effectiveness of Control

A Sample Process Control (SPC2) is included in the NeuMoDx HIV-1 Quant Assay to report process and/or amplification failures. Efficacy of this internal control was tested on the analogous NeuMoDx HCV Quant Assay under conditions representative of critical process step failures that could potentially occur during sample processing and that may not be detected by the NeuMoDx System performance monitoring sensors. Moderate positive and negative samples were run to challenge the internal control with the presence of reaction inhibitors, no NeuMoDx Wash Reagent delivery, and no wash blowout. Conditions that had an adverse effect on target detection were likewise reflected in SPC2 detection, summarized below in *Table 11*. All scenarios tested demonstrated the ability of the Sample Process Control to monitor failures adequately or that the undetected failures did not have a significant effect on target detection and quantitation.

Table 11: Summary of Sample Process Control Efficacy Study

Simulated Failure Condition	SPC2 Amplification Status	Target Amplification Status	Assay Result
Presence of Inhibitor	Not Amplified	Not Amplified	Unresolved
No Wash Reagent Delivered	Not Amplified	Not Amplified	Unresolved
No Wash Blowout	Amplified	Amplified	Positive, ± 0.3 log_{10} IU/mL of Control

Cross-Contamination

The cross-contamination rate for the NeuMoDx HIV-1 Quant Assay was determined by testing six (6) runs of alternating high positive and negative HIV-1 samples. A total of 36 negative replicates and 36 high titer HIV-1 replicates at 6.0 log_{10} IU/mL were processed in a checkerboard configuration. All replicates of the negative samples were reported negative, demonstrating the occurrence of no cross-contamination throughout sample processing on the NeuMoDx System.

Specimen Matrix Equivalence

Testing was performed to demonstrate specimen matrix equivalency between whole blood collected in EDTA and 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). Fresh specimens were kept at 2-4 °C before being spiked with four levels of HIV-1 (including a negative level) spanning the quantitative range of the NeuMoDx HIV-1 Quant Assay 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 EDTA v. ACD and fresh v. frozen plasma specimens were compared for equivalency by regression analysis. Results of the linear regression data analysis showed no significant difference in reported values between EDTA and ACD or between fresh and frozen storage conditions of plasma tested using the NeuMoDx HIV-1 Quant Assay.

Additional testing was performed to demonstrate equivalency of NeuMoDx HIV-1 Quant Assay performance on primary specimens v. secondary specimens. Panels of HIV-1 negative donor specimens spiked with HIV-1 target (AccuPlex Recombinant HIV/HCV Control) and of HIV-1 positive donor specimens were first processed from the primary specimen tubes. After primary tube processing, 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 plasma tube processing.

Clinical Method Comparison

Qualitative and quantitative performance of the NeuMoDx HIV-1 Quant Assay was compared with that of an FDA/CE-IVD cleared comparator assay. Internal testing was performed through a single-blind study of de-identified, remnant plasma specimens obtained from an FDA registered provider. A total of 723 plasma specimens were processed using the NeuMoDx HIV-1 Quant Assay on multiple NeuMoDx Systems. All samples that initially yielded an invalid result were processed again successfully, giving valid results for all specimens subject to this study.

Processing and system errors encountered during testing were minimal and well within acceptance criteria. A total of twelve (12) indeterminate (IND) results and seven (7) unresolved (UNR) results gives an indeterminate result rate of 1.48% (95% CI: 0.85-2.57%) and unresolved result rate of 0.86% (95% CI: 0.42-1.77%). The overall valid result rate was found to be 97.7% (95% CI: 96.4-98.5%).

Of the 723 valid results obtained, 165 were reported positive by the NeuMoDx HIV-1 Quant Assay with corresponding concentration values assigned by reference testing. Deming regression and Passing-Bablok regression analyses were produced to correlate reported concentration values of the NeuMoDx HIV-1 Quant Assay with the reference testing reported values.

Regression and residual plots were generated to represent the correlation between the NeuMoDx HIV-1 Quant assay concentrations and the reference test concentration values for all samples tested with concentrations assigned by both. Plots generated using the Deming method analysis and the Passing-Bablok method are shown in *Figures 5 & 6*, respectively. The quality of the Deming regression fit is illustrated by a slope coefficient of 0.975 (95% CI: 0.939, 1.011) and an intercept (bias) of -0.121 (95% CI: -0.276, 0.033), demonstrating that the concentration results obtained from the NeuMoDx HIV-1 Quant Assay and reference tests are highly correlated with acceptable bias. The quality of the Passing-Bablok linear fit is illustrated by a slope coefficient of 0.981 (95% CI: 0.950, 1.012) and an intercept (bias) of -0.167 (95% CI: -0.288, -0.036), likewise demonstrating that the concentration results obtained between the NeuMoDx HIV-1 Quant Assay and reference tests are highly correlated with acceptable bias. Results of the Deming and Passing-Bablok analyses are summarized below in *Table 12.*

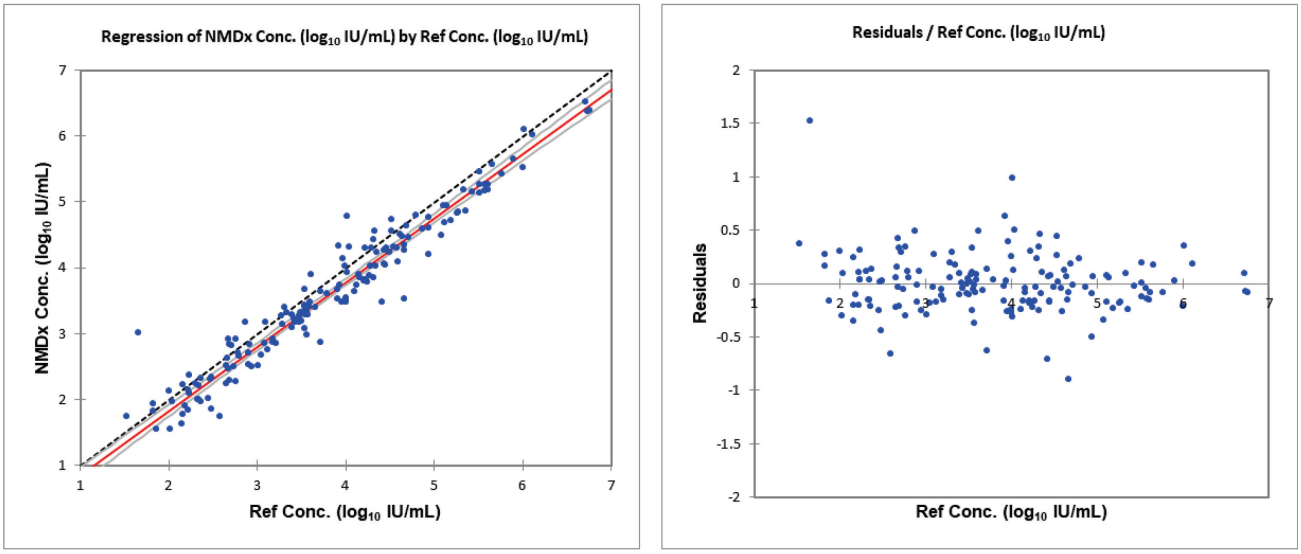

Figure 5: Equivalency (left) and Residual (right) Plots – Cumulative Analysis of the NeuMoDx HIV-1 Quant Assay v. Reference Tests – Deming Analysis

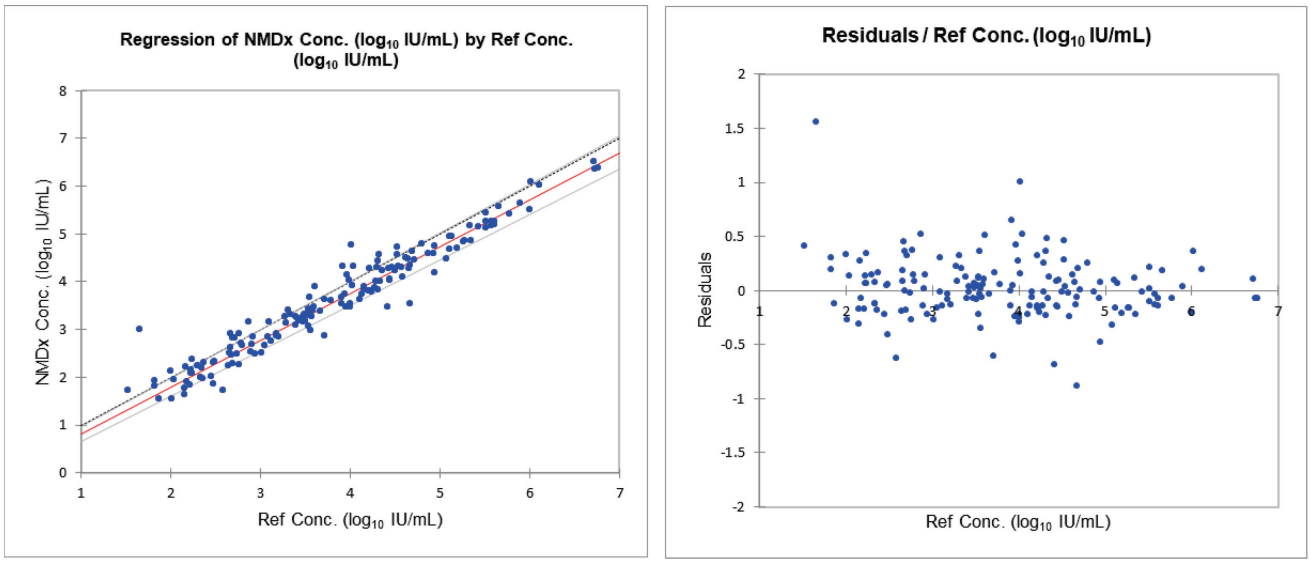

Figure 6: Equivalency (left) and Residual (right) Plots – Cumulative Analysis of the NeuMoDx HIV-1 Quant Assay v. Reference Tests – Passing-Bablok Analysis

Table 12: Summary of Deming and Passing-Bablok Linear Regression Analyses

Deming Analysis		Passing-Bablok Analysis	
Intercept	Slope Coefficient	Intercept	Slope Coefficient
-0.121 95% CI (-0.276, 0.033)	0.975 95% CI (0.939, 1.011)	-0.167 95% CI (-0.288, -0.036)	0.981 95% CI (0.950, 1.012)

Of the 723 valid results obtained using the NeuMoDx HIV-1 Quant Assay, 171 were reported positive by the reference tests and 552 were reported negative. Sensitivity and specificity of the NeuMoDx HIV-1 Quant Assay were calculated against the reference tests and are summarized below in *Table 13.* Of the 171 positive samples tested, 165 were reported positive by the NeuMoDx HIV-1 Quant Assay, demonstrating a sensitivity of 96.5% (95% CI: 92.6-98.4%). Of the 552 negative samples tested, 551 were reported negative by the NeuMoDx HIV-1 Quant Assay, demonstrating a sensitivity of 99.8% (95% CI: 99.0-100%).

Table 13: Qualitative Method Comparison Results for the NeuMoDx HIV-1 Quant Assay v. Reference Tests

		Reference Test		
	HIV-1	Positive	Negative	Total
NeuMoDx	Positive	165	1	166
	Negative	6	551	557
	Total	171	552	723
	Sensitivity = 96.5% (95% CI 92.6-98.4%)			
	Specificity = 99.8% (95% CI 99.0-100%)			

Furthermore, a total of 12 commercial seroconversion panels, including 75 individual plasma samples, were processed with the NeuMoDx HIV-1 Quant Assay to demonstrate the detection of HIV-1 RNA prior to that of antibodies/antigens using commercially available tests. Pre-seroconversion, early seroconversion, and seroconversion panel members were included in the analysis. Analysis was performed to compare the first bleed at which HIV-1 RNA is detected by the NeuMoDx HIV-1 Quant Assay in comparison to the first bleed positive for HIV-1 antibody/antigen

(Ab/Ag) as reported by commercially available FDA/CE-IVD cleared blood tests. For all panels tested, the NeuMoDx HIV-1 Quant Assay detected HIV-1 RNA at least one bleed earlier than the blood tests for Antibody/Antigen detection. Results are summarized in *Table 14.*

Table 14: Seroconversion Panel Comparison – NeuMoDx HIV-1 Quant Assay v. Blood Test for HIV-1 Ab/Ag

	Bleed Day with First Positive Result	
Panel ID	**NeuMoDx HIV-1 Quant Assay**	**HIV-1 Ab/Ag Blood Test**
PRB969	**4**	7
PRB968	**5**	7
0600-0230	**2**	4
0600-0270	**2**	3
0600-0258	**2**	3
0600-0244 (PRB962)	**3**	5
0600-0272	**3**	4
PRB967	**2**	4
PRB964	**3**	6
PRB963	**4**	6
0600-0263	**5**	7
PRB956	**2**	4

Additional analyses were performed to compare the first bleed at which HIV-1 RNA is detected by the NeuMoDx HIV-1 Quant Assay with the first bleed positive for HIV-1 RNA as revealed by commercially available FDA/CE-IVD cleared NAT tests. For all panels tested, the NeuMoDx HIV-1 Quant Assay detected HIV-1 RNA at the same bleed as the other NAT tests for HIV-1 RNA detection. In two panels, the NeuMoDx HIV-1 Quant Assay demonstrated HIV-1 RNA detection one bleed earlier than other NAT tests. Results are summarized in *Table 15*.

Table 15: Seroconversion Panel Comparison – NeuMoDx HIV-1 Quant Assay v. NAT for HIV-1 RNA

	Bleed Day with First Positive Result	
Panel ID	**NeuMoDx HIV-1 Quant Assay**	**Reference NAT**
PRB969	4	4
PRB968	5	5
0600-0230	2	2
0600-0270	2	2
0600-0258	2	2
0600-0244 (PRB962)	3	3
0600-0272	3	3
PRB967	2	2
PRB964	**3**	4
PRB963	**4**	5
0600-0263	5	5
PRB956	2	2

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TRADEMARKS

SYMBOLS

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

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