REF 201400 NeuMoDx™ CMV 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 CMV Quant Assay is an automated, *in vitro* nucleic acid amplification test for the quantitation of cytomegalovirus (CMV) DNA in human plasma specimens for CMV genotypes gB1 through gB4 of CMV-infected individuals. The NeuMoDx CMV Quant Assay implemented on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx System(s)) incorporates automated DNA extraction to isolate the target nucleic acid from the specimen and real-time polymerase chain reaction (PCR) to target the highly conserved sequences in the cytomegalovirus genome.

The NeuMoDx CMV Quant Assay is intended for *in vitro* quantitation of cytomegalovirus (CMV) DNA in fresh and frozen human plasma specimens using NeuMoDx 288 and NeuMoDx 96 Molecular Systems. This assay is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management and monitoring of CMV infection. The assay is not intended for use as a screening test for the presence of CMV in blood or blood products.

SUMMARY AND EXPLANATION

Human whole blood collected in sterile blood collection tubes containing either EDTA or ACD as anticoagulation agent may be used for the preparation of plasma. To prepare for testing, plasma, in a 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 550 µL aliquot of the plasma sample is mixed with NeuMoDx Lysis Buffer 1 and the NeuMoDx System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the products of amplification (sections of the CMV genome target in highly conserved regions). The NeuMoDx CMV Quant Assay includes a DNA Sample Process Control (SPC1) to help monitor for the presence of potential inhibitory substances as well as NeuMoDx System or reagent failures that may be encountered during the extraction and amplification process.

CMV is a common double-stranded DNA virus of the human herpesvirus family that infects people of all ages. It is estimated that by age 40, more than half of the population will have been infected with CMV.[1] CMV is spread through body fluids such as saliva, urine, blood, tears, semen and breastmilk. Immunocompetent individuals infected with CMV are typically asymptomatic, but infection with the virus can be serious in babies and people with weakened immune systems. Pregnant mothers can pass on CMV to their unborn children and cause congenital CMV which may result in hearing loss among other developmental and motor delays. CMV is a major pathogen for immunocompromised patients, including solid organ transplant recipients, hematopoietic cell transplant recipients, HIV-infected patients, and patients treated with immunomodulating drugs.[2] CMV viral load monitoring is primarily used in these immunocompromised populations, where it causes many morbidities including pneumonia, gastrointestinal tract disease, hepatitis, and encephalitis as well as increasing the chance of organ rejection and other opportunistic infections.

Diagnosis of CMV infection is not based on nucleic acid testing (NAT) alone; NAT testing is used in addition to antigen testing which involves the staining of polymorphonuclear leukocytes (PMNs) for early structural lower matrix protein of CMV as well as other symptoms the patient may be experiencing. CMV viral load testing is routinely used to determine when anti-viral therapy is necessary as well as to monitor the effectiveness of such therapies.[3] While, current guidelines for the management and treatment of CMV infections in immunocompromised individuals are ambiguous in terms of *when* to start anti-viral therapy, they all require constant viral load monitoring once anti-viral therapy is initiated to aid in mitigating the severe side effects of medications in such populations.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx CMV Quant Assay on the NeuMoDx System utilizes the NeuMoDx CMV Quant Test Strip, NeuMoDx CMV Calibrators, NeuMoDx CMV External Controls, NeuMoDx Lysis Buffer 1, and NeuMoDx general use reagents to perform the analysis. The NeuMoDx CMV Quant Assay combines automated DNA extraction, amplification and detection by real-time PCR. Whole blood specimens are collected in EDTA or ACD tubes, for the preparation of plasma. The plasma specimen in a NeuMoDx System compatible specimen tube is placed into a Specimen Tube Carrier, which is then loaded onto the NeuMoDx System for processing. No further operator intervention is necessary.

The NeuMoDx Systems use a combination of heat, lytic enzyme, and extraction reagents to automatically perform cell lysis, DNA extraction and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles, with the bound nucleic acids, are loaded into the NeuMoDx Cartridge where the unbound, non-DNA components are further washed away with NeuMoDx Wash Reagent and the bound DNA is eluted using NeuMoDx Release Reagent. The NeuMoDx Systems then use the eluted DNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for PCR amplification of the CMV-specific and SPC1 targets. Upon reconstitution of the NeuDry PCR reagents, the NeuMoDx System dispenses the prepared, PCR ready mixture into the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences (if present) occur in the PCR chamber area of the NeuMoDx Cartridge. The NeuMoDx Cartridge is also designed to contain the amplicon following real-time PCR and essentially eliminate contamination risk post-amplification.

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 for 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, resulting in the quencher molecule quenching the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan probes are designed such that they anneal within an DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore and breaks the close proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing fluorescence detection of the fluorophore. The resulting fluorescent signal detected in the NeuMoDx System quantitative PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target DNA 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 CMV DNA. For detection of the SPC1, the TaqMan probe is labeled with an alternate fluorescent dye (Excitation: 535 nm & Emission: 556 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software analyzes the data and reports a final result (POSITIVE / NEGATIVE / INDETERMINATE / UNRESOLVED). If a result is POSITIVE, the NeuMoDx System software also provides a quantitative value associated with the sample or reports if the calculated concentration is within the limits of quantitation.

REAGENTS/CONSUMABLES

Material Provided

REF	Contents	Tests per unit	Tests per package
201400	**NeuMoDx CMV Quant Test Strip** *Dried PCR reagents containing CMV specific TaqMan probes and primers, SPC1 specific TaqMan probe and primers.*	16	96

Reagents and Consumables Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, Lytic enzyme, and sample process controls*
800400	**NeuMoDx CMV Calibrators** *Single use sets of CMV High and Low Calibrators to establish validity of standard curve*
900401	**NeuMoDx CMV External Controls** *Single use sets of CMV Positive and Negative Controls to establish daily validity of NeuMoDx CMV Quant Assay*
400400	**NeuMoDx Lysis Buffer 1**
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100100	**NeuMoDx Cartridge**
235903	**Hamilton CO-RE / CO-RE II Tips (300 µL) with Filters**
235905	**Hamilton CO-RE / CO-RE II Tips (1000 µL) with Filters**

Instrumentation Required

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

WARNINGS & PRECAUTIONS

- The NeuMoDx CMV 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 CMV Calibrators [REF 800400]) must be available before test results can be generated for clinical samples.
- NeuMoDx CMV External Controls [REF 900401] must be processed every 24 hours throughout testing with the NeuMoDx CMV Quant Assay.

- Minimum specimen volume is 1 mL of EDTA/ACD plasma when using the 32-tube carrier; volume less than 1 mL may result in a NeuMoDx System error.
- Performing a CMV assay on specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results when using the NeuMoDx CMV Quant Test Strip.
- Avoid microbial and deoxyribonuclease (DNase) contamination of all reagents and consumables at all times. The use of sterile DNase-free disposable transferring pipettes is recommended. 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 CMV 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 CMV Quant Test Strip or NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 1; handling of the consumables and reagents should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are available upon request.
- 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*[4] and in CLSI Document M29-A4.[5]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

PRODUCT STORAGE, HANDLING & STABILITY

- All NeuMoDx reagents and consumables (with the exception of external controls and calibrators) are stable in the primary packaging at 18 to 23°C through the stated expiration date on the immediate product label.
- A NeuMoDx CMV Quant Test Strip loaded into the NeuMoDx System is stable for 14 days; the NeuMoDx System software will prompt the removal of the test strips that have been in-use on board the NeuMoDx System for longer than 14 days and new NeuMoDx CMV Quant Test Strips will need to be opened and loaded on the NeuMoDx System.
- The NeuMoDx calibrators and controls are non-infectious but should be discarded in laboratory biohazard waste after use as they will contain target material after processing on the system which may cause contamination if not handled properly.

SPECIMEN COLLECTION, TRANSPORT & STORAGE

1. Handle all specimens 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.
4. Whole blood collected in devices listed above may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation. Plasma preparation should be performed according to manufacturer instructions.
5. Prepared plasma specimens may remain 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.
6. Prepared plasma specimens should be stored between 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 $\leq$ -20°C for up to 26 weeks for plasma before processing; plasma samples should not be subjected to more than 2 freeze/thaw cycles prior to use.
    a. If samples are frozen, allow the samples to completely thaw at room temperature (15 - 30°C); vortex to generate a uniformly distributed sample.
    b. Once frozen samples are thawed, testing should occur within 8 hours.
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 CMV testing.
10. Proceed to *Test Preparation* section.

The overall process for implementation of the NeuMoDx CMV Quant Assay is summarized below in *Figure 2*.

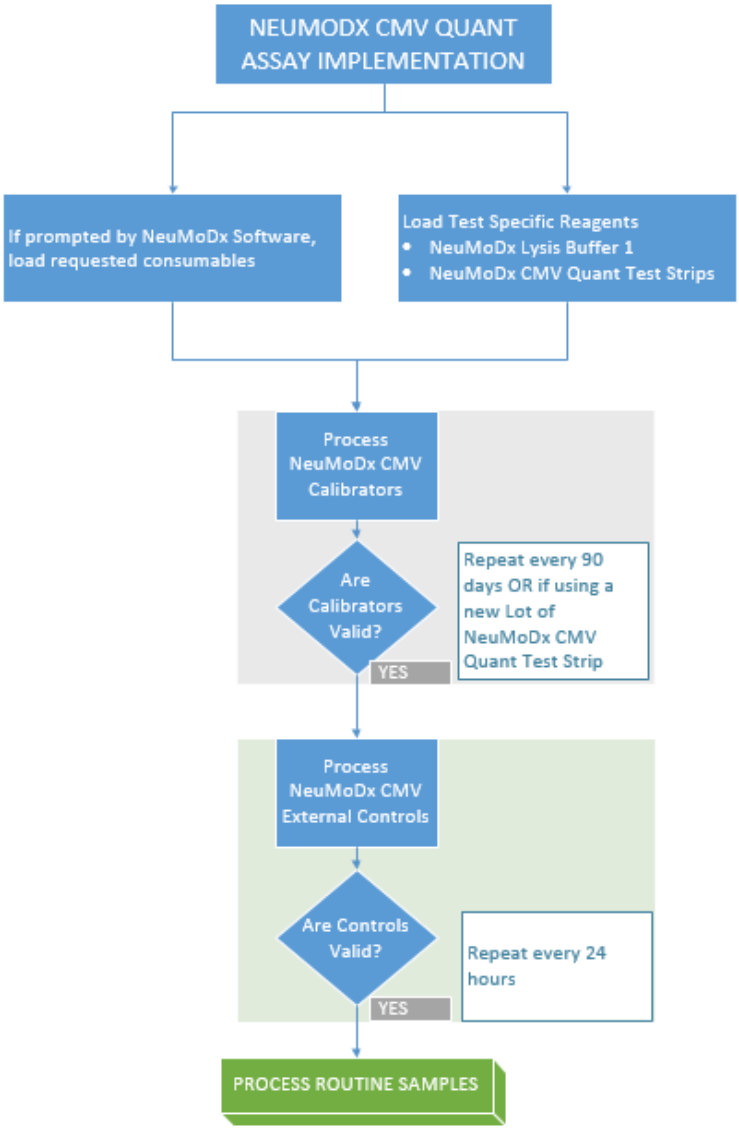

Figure 1: NeuMoDx CMV Quant Assay Implementation Workflow

INSTRUCTIONS FOR USE

Test Preparation

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
2. Using a transfer pipette, transfer ≥ 1 mL of plasma to the barcoded specimen (secondary) tube if using the 32-Tube Carrier or >2mL if using the 24-Tube Carrier. Care should be taken not to transfer any clots from the plasma sample into the specimen tube. Use a different transfer pipette for each specimen.
3. The secondary tube must meet the following tube specifications compatible with the NeuMoDx System based on Specimen Tube Carrier being used for processing.
   - 32-Tube Carrier: between 11 mm and 14 mm in diameter and between 60 mm and 120 mm in height
   - 24-Tube Carrier: between 14.5 mm and 18 mm in diameter and between 60 mm and 120 mm in height

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 CMV 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, or Biohazardous Waste Container as appropriate.
4. If prompted by the NeuMoDx System software, process the calibrators [REF 800400] and/or external controls [REF 900401] as required. Further information regarding calibrators and controls can be found in the *Results Processing* section.
5. Load the specimen/calibrator/control tube(s) into a standard 32-Tube Carrier and ensure caps are removed from all specimen tubes.
6. Place the Specimen Tube Carrier in any open position on the Autoloader shelf and use the touchscreen to load carrier into the NeuMoDx System. This will initiate processing of the loaded specimens for the test(s) identified.

LIMITATIONS

- The NeuMoDx CMV Quant Test Strip can only be used on NeuMoDx Systems.
- The performance of the NeuMoDx CMV Quant Test Strip has been established for plasma specimens prepared from whole blood collected with EDTA/ACD as anti-coagulant; the use of the NeuMoDx CMV Quant Test Strip with other clinical specimen types has not been assessed and performance characteristics of the test are unknown for other specimen types.
- Since detection of CMV is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- Calibrators and external controls must be processed as recommended in the package inserts and if prompted by NeuMoDx System software before processing routine clinical samples.
- 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 CMV Quant Assay.
- Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
- If both the CMV target and the SPC1 target do not amplify, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
- If the NeuMoDx CMV Quant Assay result is Positive, but the quantitation value is beyond the limits of quantitation, the NeuMoDx System will report whether the detected CMV was *below* Lower Limit of Quantitation (LLoQ) or *above* Upper Limit of Quantitation (ULoQ).
- In the event the detected CMV was below LLoQ, the NeuMoDx CMV Quant Assay may be repeated (if desired) with another aliquot of the specimen.
- In the event the detected CMV is above ULoQ, the NeuMoDx CMV Quant Assay may be repeated with a diluted aliquot of the original specimen. A 1:100 or 1:1000 dilution in CMV negative plasma or Basematrix 53 Diluent (Basematrix) (SeraCare, Milford, MA) is recommended. The concentration of the original specimen can be calculated as follows:

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

- The occasional presence of PCR inhibitors in plasma may result in a system Quantitation Error; if this occurs, it is recommended to repeat the test with the same specimen diluted in Basematrix at 1:10 or 1:100.
- A positive result does not necessarily indicate the presence of viable organisms. However, a positive result is presumptive for the presence of cytomegalovirus DNA.
- Deletion or mutations in the conserved regions targeted by the NeuMoDx CMV Quant Assay may affect detection or could lead to an erroneous result using the NeuMoDx CMV Quant Test Strip.
- Results from NeuMoDx CMV 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.
- 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 CMV Quant Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx CMV Assay Definition File (CMV ADF). A NeuMoDx CMV Quant Assay result may be reported as Negative, Positive with a reported CMV concentration, Positive above ULoQ, Positive below LLoQ, Indeterminate or Unresolved based on the amplification status of the target and sample processing control. Results are reported based on the decision algorithm in *Table 1*.

Table 1: NeuMoDx CMV Quant Assay Decision Algorithm

Result	CMV	Sample Process Control (SPC1)
Positive	[2 ≤ Ct ≤ 9 AND EPR > 2 AND EP ≥ 1500] OR [9 ≤ Ct ≤ 41 AND EP ≥ 1500]	N/A
Positive, above Upper Limit of Quantitation [ULoQ] (log_{10} IU/mL)	[CONC] > 8.0 log_{10} IU/mL, NO QUANT	N/A
Positive, below Lower Limit of Quantitation [LLoQ] (log_{10} IU/mL)	[CONC] < 1.3 log_{10} IU/mL, NO QUANT	N/A
Negative	N/A OR [2 ≤ Ct <9 AND EPR ≤ 2] OR [9 ≤ Ct ≤ 41 AND EP < 1500] OR Ct > 41	AMPLIFIED (28 ≤ Ct ≤ 34) and EP ≥ 2000
Indeterminate	NOT AMPLIFIED/ Systems Errors Noted	
Unresolved	NOT AMPLIFIED/ No System Errors Noted	

EP = End Point Fluorescence (after baseline correction); EPR = End Point Fluorescence Ratio; C_t = Cycling Threshold;
Quant = calculated quantity of CMV present expressed in log_{10} IU/mL. See Test Calculation below.

Test Calculation

1. For samples within the Quantitation range of the NeuMoDx CMV Quant Assay, the concentration of CMV DNA 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 CMV calibrators processed to establish validity of the Standard Curve, for a particular lot of the NeuMoDx CMV Quant Test Strip, on a specific NeuMoDx System.
    b. The calibration coefficient is incorporated into the final determination of the concentration of CMV DNA.
2. NeuMoDx CMV Quant Assay results are reported in log_{10} IU/mL.
3. The resulting quantitation of the unknown samples is traceable to the WHO 1st CMV International Standard.

Test Calibration

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

External Calibrators

1. NeuMoDx CMV Calibrators are provided in a kit [REF 800400] and contain non-infectious encapsulated CMV target prepared in Basematrix.
2. A set of CMV calibrators need to be processed with each new lot of NeuMoDx CMV Quant Test Strips, or if a new CMV Assay Definition File is uploaded to the NeuMoDx System, or 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 as to when the 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 - high and low – need to be processed to establish validity.
    b) To generate valid results, at least 2 out of the 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 CMV 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 a second consecutive time, 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. External control materials, which contain non-infectious encapsulated CMV target in Basematrix for positive controls, are provided by NeuMoDx Molecular, Inc. in a kit containing the NeuMoDx CMV External Controls [REF 900401].
2. Positive and negative external controls need to be processed once every 24 hours. If a set of valid external controls does not exist, the NeuMoDx System software will prompt the user for these controls to be processed before sample results can be reported.
3. If external controls are required, retrieve the set of external controls from freezer and allow the vials to set at room temperature (15-30°C) until completely thawed. Vortex gently to ensure homogeneity.
4. Using the touchscreen and a Specimen Tube Carrier placed on the Autoloader shelf, load the positive and negative control vials into the NeuMoDx System. The NeuMoDx System will recognize the barcode and begin processing the specimen tubes unless reagents or consumables required for testing are not available.
5. Validity of external controls will be assessed by the NeuMoDx System based on the expected result. The positive control should provide a CMV Positive result and the negative control should provide a CMV Negative result.
6. 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, repeat the failed NeuMoDx CMV external control(s) with a freshly thawed vial of the control(s) failing the validity test.
    d) If positive NeuMoDx CMV external control continues to report a Negative result, contact NeuMoDx customer service.
    e) If negative NeuMoDx CMV external control continues to report a Positive result, attempt to eliminate all sources of potential contamination, including replacing ALL reagents before contacting NeuMoDx customer service.

Sample Process (Internal) Controls

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

Invalid Results

If a NeuMoDx CMV 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.

An UNR result will be reported if no valid amplification of CMV DNA or SPC1 is detected, which indicates possible reagent failure or the presence of inhibitors. In the event a UNR result is reported, a retest may be performed as a first step. If a retest fails, a diluted specimen may be used to mitigate the effects of any sample inhibition.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity – Limit of Detection using the WHO Standard

The Analytical Sensitivity of the NeuMoDx CMV Quant Assay was characterized by testing negative specimens and a dilution series of the WHO 1st International Standard in screened negative human plasma 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 processed 18 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 CMV Quant Assay

Target Concentration [IU/mL]	Target Concentration [log_{10} IU/mL]	PLASMA		
		Number of Valid Tests	Number of Positives	Detection Rate
50	1.70	108	108	100.0%
30	1.48	108	107	99.1%
25	1.40	108	106	98.1%
20	1.30	108	105	97.2%
15	1.18	108	99	91.7%
NEG	---	108	0	0.0%

The LoD of the NeuMoDx CMV Quant Assay in plasma for the variant gB1 was determined to be 17.7 IU/mL (1.25 log_{10} IU/mL) with 95% Confidence Interval (CI) of 13.8 - 21.0 IU/mL, (1.14 - 1.32 log_{10} IU/mL) [*Figure 3*]. The LoD across genotypes is 20.0 IU/mL (1.30 log_{10} IU/mL) as determined by hit rate analysis.

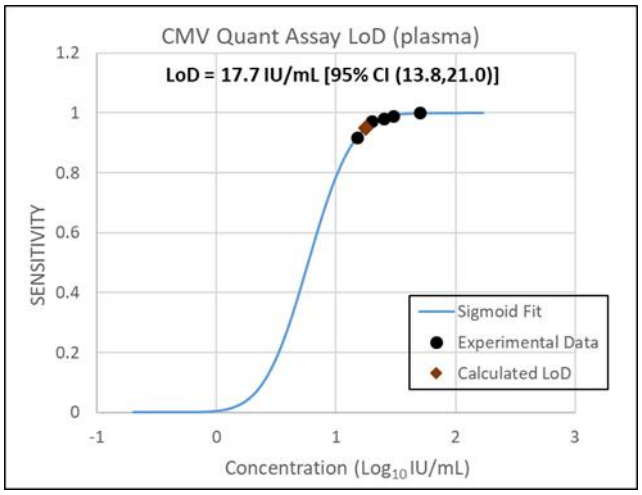

Figure 2: Probit Style Analysis Used to Determine the LoD of the NeuMoDx CMV Quant Assay in Plasma Samples

Analytical Sensitivity – Quantitation Limit – Lower Limit of Quantitation (LLoQ)

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

TAE = bias + 2*SD (Westgard Statistic)

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

Compiled results for the 5 levels of CMV (variant gB1) plasma specimens used in the LLoQ study are shown in *Table 3*. Based on this data set and the previously determined LoD, the LLoQ was determined to be 20.0 IU/mL (1.30 log_{10} IU/mL) and confirmed across genotypes.

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

Target Conc. [IU/mL]	Target Conc. [log_{10} IU/mL]	Plasma				
		Average Conc. [log_{10} IU/mL]	Detection (%)	SD	Bias	TAE
50	1.70	1.75	100.0	0.16	0.05	0.37
30	1.48	1.62	99.1	0.24	0.14	0.62
25	1.40	1.56	98.1	0.19	0.17	0.55
20	1.30	1.57	97.2	0.22	0.27	0.72
15	1.18	1.52	91.7	0.21	0.35	0.78

Based on the outcome of these studies, the LoD and LLoQ of of the NeuMoDx CMV Quant Assay were both determined to be 20.0 IU/mL [1.30 log_{10} IU/mL].

Linearity and Determination of Upper Limit of Quantitation (ULoQ)

Linearity and the Upper Limit of Quantitation (ULoQ) of the NeuMoDx CMV Quant Assay were established in plasma by preparing a dilution series using the NeuMoDx encapsulated CMV target and Exact CMV Positive Control (Exact Diagnostics, Fort Worth, TX) with established traceability to the 1st WHO International Standard. A 9-member panel was prepared in pooled CMV negative plasma to create a panel that would span a concentration range of 8 – 1.7 log_{10} IU/mL. . The ULoQ of the NeuMoDx CMV Quant Assay was determined to be 8.0 log_{10} IU/mL. The CMV assay concentrations reported by the NeuMoDx System compared to the expected values are presented in *Figure 4.*

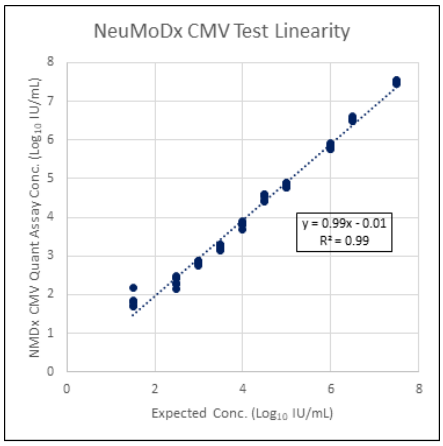

Figure 3: Linearity of the NeuMoDx CMV Quant Assay

Linearity Across Genotypes

The linearity of the NeuMoDx CMV Quant Assay across four CMV genotypes (gB1, gB2, gB3 and gB4) was characterized by testing five different concentrations of each genotype of CMV prepared in pooled CMV-negative plasma. The levels of CMV targets tested in this study were dependent on the concentration of the source specimen, and therefore differed across genotypes. The study was performed by testing 6 replicates of each of 4 genotypes at 5 concentrations. The linearity across four CMV genotypes is presented in *Table 4* and *Figure 5*.

Table 4: Linearity of the NeuMoDx CMV Quant Assay Across Genotypes

Genotype	Linearity Equation y = NeuMoDx CMV Assay Quantitation x = Expected Quantitation	R^2
gB1	y = 0.960x + 0.103	0.994
gB2	y = 0.989x + 0.009	0.996
gB3	y = 1.023x + 0.099	0.967
gB4	y = 0.968x + 0.004	0.992

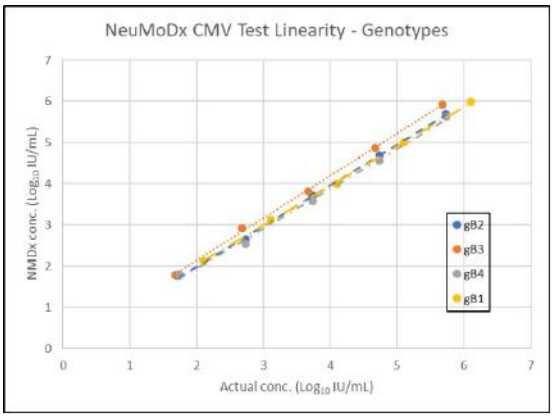

Figure 4: Linearity of the NeuMoDx CMV Quant Assay across Genotypes

Analytical Specificity – Cross-Reactivity

Analytical specificity was demonstrated by screening 35 organisms commonly found in blood/plasma specimens as well as species phylogenetically similar to CMV for cross-reactivity**.** Organisms were prepared in pools of between 5 – 6 organisms and tested at a high concentration. The organisms tested are shown in *Table 5*. No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx CMV Quant Assay.

Table 5: Pathogens Used to Demonstrate Analytical Specificity

Non-Target Organisms					
BK Polyomavirus	Adenovirus type 5	Herpes Simplex Virus type-1	*Clostridium perfringens*	*Mycoplasma pneumoniae*	*Streptococcus pneumoniae*
Epstein-Barr Virus	Hepatitis C Virus	Herpes Simplex Virus type-2	*Enterococcus faecalis*	*Neisseria gonorrhoeae*	*Streptococcus pyogenes*
Human Herpes Virus type-6	Parvovirus B19	Varicella-Zoster Virus	*Escherichia coli*	*Propionibacterium acnes*	*Aspergillus niger*
Human Herpes Virus type-7	JC Virus	HIV 1	*Klebsiella pneumoniae*	*Salmonella typhimurium*	*Candida albicans*
Human Herpes Virus type-8	Human Papillomavirus 16	HIV 2	*Listeria monocytogenes*	*Staphylococcus aureus*	*Cryptococcus neoformans*
Hepatitis B Virus	Human Papillomavirus 18	*Chlamydia trachomatis*	*Mycobacterium avium*	*Staphylococcus epidermidis*	

Analytical Specificity – Interfering Substances, Commensal Organisms

The NeuMoDx CMV 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 5*. Negative CMV plasma was spiked with the organisms pooled in groups of 4-7, and also spiked with CMV target at a concentration of 3 $\log_{10}$ 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 CMV Quant Assay was evaluated in the presence of typical exogenous and endogenous interfering substances encountered in CMV clinical plasma specimens. These included abnormally high levels of blood components as well as common antiviral medications, which were classified in *Table 6*. Each substance was added to screened CMV-negative human plasma spiked with 3 $\log_{10}$ IU/mL CMV and samples were analyzed for interference. In addition, common disease state plasma associated with CMV infection were also tested for potential interference. The average concentration and bias of all substances tested as compared to control samples spiked with same level CMV are reported in *Table 7.* None of the exogenous and endogenous substances affected the specificity of the NeuMoDx CMV Quant Assay.

Table 6: Interference Testing - Exogenous Agents (Drug Classifications)

Pool	Drug name	Classification	Pool	Drug name	Classification
Pool 1	Azathioprine	Immunosuppressant	Pool 4	Trimethoprim	Antibiotic
	Cyclosporine	Immunosuppressant		Vancomycin	Antibiotic
	Foscarnet	Antiviral (Herpesviridae)		Tacrolimus	Immunosuppressant
	Ganciclovir	Antiviral (CMV)		Everolimus	Immunosuppressant
	Valganciclovir hydrochloride	Antiviral (CMV)		Clavulanate potassium	Antibiotic
Pool 2	Prednisone	Corticosteroid/Immunosuppressant	Pool 5	Famotidine	Histamine receptor antagonist
	Cidofovir	Antiviral (CMV)		Sulfamethoxazole	Antibiotic
	Cefotetan	Antibiotic (broad spectrum)		Valacylovir	Antiviral (Herpesviridae)
	Cefotaxime	Antibiotic (broad spectrum)		Letermovir	Antiviral (CMV)
	Fluconazole	Antifungal		Ticarcillin disodium	Antibiotic
Pool 3	Mycophenolate mofetil	Immunosuppressant		Leflunomide	Immunosuppressant
	Mycophenolate sodium	Immunosuppressant			
	Piperacillin	Antibiotic			
	Sirolimus/ Rapamycin	Immunosuppressant)			
	Tazobactam	Modified antibiotic			

Table 7: Interference Testing - Exogenous and Endogenous Agents

Endogenous	**Average Conc.**	**Bias**
	log_{10} IU/mL	**log_{10} IU/mL**
Hemoglobin	2.97	0.07
Triglycerides	3.03	0.13
Bilirubin	3.01	0.11
Albumin	2.88	-0.02
Exogenous (Medications)	**Average Conc.**	**Bias**
	log_{10} IU/mL	**log_{10} IU/mL**
Pool 1: Azathioprine, Cyclosporine, Foscarnet, Ganciclovir, Valganciclovir hydrochloride	2.88	-0.02
Pool 2: Prednisone, Cidofovir, Cefotetan, Cefotaxime, Fluconazole	2.91	0.01
Pool 3: Mycophenolate mofetil, Mycophenolate sodium, Piperacillin, Sirolimus/Rapamycin, Tazobactam	2.98	0.08
Pool 4: Trimethoprim, Vancomycin, Tacrolimus, Everolimus, Clavulanate potassium	3.05	0.15
Pool 5: Famotidine, Sulfamethoxazole, Letermovir, Valacyclovir, Ticarcillin disodium, Leflunomide	2.87	-0.03
Disease State	**Average Conc.**	**Bias**
	log_{10} IU/mL	**log_{10} IU/mL**
Antinuclear Antibody (ANA)	2.90	0.00
Systemic Lupus Erythematosus (SLE)	3.04	0.14
Rheumatoid Arthritis	2.99	0.09

Within Lab Precision

Precision of the NeuMoDx CMV Quant Assay was determined by testing 3 replicates of a 4-member panel of CMV specimens prepared with Exact CMV Positive Control (Exact Diagnostics, Fort Worth, TX) twice a day, using two NeuMoDx 288 Systems and one NeuMoDx 96 System across 12 days. 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. Excellent precision was demonstrated across systems, days, or runs as shown in *Table 8*. Precision between operators was not characterized as the operator plays no significant role in the processing of samples using the NeuMoDx System.

Table 8: Within Lab Precision – NeuMoDx CMV Quant Assay on NeuMoDx Systems

Target CMV Conc. [log_{10} IU/mL]	Average CMV Conc. [log_{10} IU/mL]	Within System SD	Within Day SD	Within Run SD	Overall (Within Lab) SD
5.7	5.64	0.09	0.09	0.07	0.13
4.7	4.58	0.10	0.10	0.08	0.14
3.7	3.60	0.09	0.09	0.07	0.12
2.7	2.62	0.13	0.13	0.10	0.15

Lot to Lot Reproducibility

Lot to Lot Reproducibility of the NeuMoDx CMV Quant Assay was determined using three different lots of key reagents – NeuMoDx Lysis Buffer 1, NeuMoDx Extraction Plates and the NeuMoDx CMV Quant Test Strips. A 4-member panel of CMV prepared with Exact CMV 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 9*. Maximum overall bias was 0.12 log_{10} IU/mL and maximum overall SD was 0.39 log_{10} IU/mL. Equivalent performed was demonstrated across lots as quantitation of all panel members was within tolerance specification.

Table 9: Lot to Lot Reproducibility – NeuMoDx CMV Quant Assay

Target CMV Conc. [log_{10} IU/mL]	Average CMV Conc. [log_{10} IU/mL]	N (Valid Results Per Lot)	Bias	Between Lot SD	Within Lot SD	Overall SD
5.7	5.65	36	0.05	0.27	0.15	0.31
4.7	4.63	36	0.07	0.22	0.13	0.26
3.7	3.58	36	0.12	0.34	0.18	0.39
2.7	2.64	36	0.06	0.12	0.14	0.18

Effectiveness of Control

The SPC1 is included in the NeuMoDx CMV 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 (at 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 solution delivered, and no wash blow out. Process inefficiencies that had an adverse effect on CMV detection/quantitation were mirrored by performance of SPC1 target as shown in *Table 10*. 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 SPC1 detection nor CMV detection and quantitation. Therefore, the SPC1 demonstrated success in effectively monitoring assay performance on the NeuMoDx System.

Table 10: Effectiveness of the Sample Process Control

Process Step Failure Tested	Sample Process Control 1 Amplification Status	CMV 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 CMV Assay on the NeuMoDx Systems was used for determination of the percentage of valid results. Valid test results are reported as 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 1,100 individual NeuMoDx CMV Quant Assay results included in the retrospective analysis, which included data obtained on both the NeuMoDx 288 and NeuMoDx 96 Systems. The UNR rate was determined to be 0.91% (10/1100) and the IND rate was determined to be 0.36% (4/1100); which meet the acceptance criteria of the analysis. Therefore, the valid result rate of the NeuMoDx CMV Assay across NeuMoDx Systems was concluded to be 98.7% with 95% CI (97.9- 99.2).

Cross-contamination

The cross-contamination rate for NeuMoDx CMV Quant Assay was determined by testing three sets of CMV specimens featuring alternating high positive and negative specimens. In total, this involved testing 108 replicates of CMV-negative plasma and 108 replicates of a spiked CMV plasma at 6.0 log10 IU/mL. All 108 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). Fresh specimens were kept at 4°C until they were spiked with three levels of CMV 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 vs. frozen plasma as well as EDTA vs ACD plasma specimens were compared for equivalency by regression analysis. The data demonstrated excellent equivalency between EDTA and ACD plasma specimens, and fresh and frozen plasma specimens with slopes within 0.02 of 1.0 and very low bias (intercept), as presented in *Table 11* below.

Table 11: Specimen Matrix Equivalency

Parameter Requirement	ACD vs K2EDTA		Fresh vs Frozen	
	Fresh	Frozen	ACD	EDTA
Slope [0.9-1.1]	1.000	0.982	1.014	1.000
Intercept [<0.5 $\log_{10}$ IU/mL]	-0.050	0.018	-0.061	0.020
p-value > 0.05	0.848	0.644	0.895	0.631

Clinical Method Comparison

Quantitative performance of the NeuMoDx CMV Quant Assay was assessed against FDA/CE-approved comparator assays by testing undiluted clinical specimens from CMV infected patients. Testing was performed internally at NeuMoDx through a single-blinded study of de-identified, residual, clinical specimens obtained from four external reference laboratories. A total of 284 plasma specimens were processed using NeuMoDx CMV Quant Assay in a (single) blinded manner across multiple NeuMoDx Molecular Systems.

The processing and system errors obtained across the NeuMoDx Molecular Systems were minimal and met the criteria. A total of 3 Indeterminate (IND) results were obtained for the samples which resulted in an overall initial IND rate of 1% with 95% CI (0.27 -3.32 %). There was insufficient volume to reprocess these 3 specimens under the normal workflow. There were 10 Unresolved (UNR) results initially obtained but following CMV Quant Assay recommended procedure for a 1:10 dilution in Basematrix for UNR results, valid results were obtained upon repeat testing of all 10 of the UNR samples diluted apppropriately, Therefore, the Total Processing Error Rate was 1.06% with 95% CI (0.27% - 3.3%) due to the Indeterminate results that were unable to be repeat tested due to insufficient volume.

There were 4 samples that generated a Quantitation Error flag and 3 of those 4 were able to be repeat tested per the recommonded procedure using a 1:10 dilution of the sample in Basematrix in order to obtain a valid quantitave result. Of the 283 valid results obtained in the study, 129 samples were reported Positive by the NeuMoDx CMV Assay with corresponding concentration values assigned by the reference tests. For six of those samples, five were reported below LLoQ and one was reported above ULoQ by the reference test and therefore, a total of 123 samples had corresponding concentration values assigned by both the NeuMoDx CMV Quant Assay and the reference CE-IVD tests and were used for quantitative correlation analysis. Demming Regression and Passing-Bablok Regression analyses were used to correlate between the concentration values of the NeuMoDx CMV Assay and the values reported by the reference tests.

Equivalency plots were generated to represent the correlation between the NeuMoDx CMV Quant Assay concentrations and the reference tests concentration values for all samples tested using the Deming Regression fit and Passing-Bablok fit and are presented *Figure 6*.

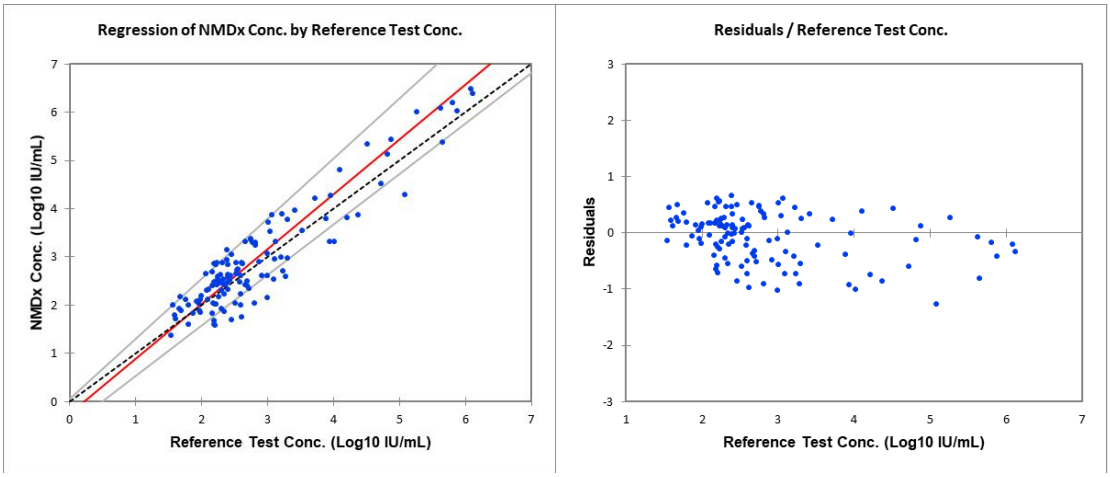

Figure 5: Equivalency *(left)* and Residual *(right)* Plots – Cumulative Analysis (across both NeuMoDx Systems) of NeuMoDx CMV Quant Assay Results Compared to Reference Test Results for ALL samples based on Passing-Bablok Regression Analysis.

The quality of the Deming Regression fit is illustrated by a slope coefficient of 1.1 with a 95% CI (1.0, 1.2), and an intercept (bias) of -0.18 with a 95% CI -0.39, 0.03), demonstrating that the concentration results obtained between the NeuMoDx CMV 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 1.1 with a 95% CI (1.0, 1.2), and an intercept (bias) of -0.24 with a 95% CI (-0.51, 0.06), demonstrating that the concentration results obtained between the NeuMoDx CMV Quant Assay and Reference tests are highly correlated and with acceptable bias as shown in *Table 12.*

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

Deming Analysis		Passing-Bablok Analysis	
Intercept	**Slope Coefficient**	**Intercept**	**Slope Coefficient**
-0.18 95%CI (-0.39, 0.03)	1.1 95%CI (1.0, 1.2)	-0.24 95%CI (-0.51, 0.06)	1.1 95%CI (1.0, 1.2)

REFERENCES

1. Centers for Disease Control (CDC). Cytomegalovirus (CMV) and Congenital CMV Infection. (2018). Retrieved from https://www.cdc.gov/cmv/clinical/features.html
2. Kraft, C. S., Armstrong, W. S., & Caliendo, A. M. (2012). Interpreting quantitative cytomegalovirus DNA testing: understanding the laboratory perspective. Clinical infectious diseases, 54(12), 1793-1797.
3. A Ross, S., Novak, Z., Pati, S., & B Boppana, S. (2011). Overview of the diagnosis of cytomegalovirus infection. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 11(5), 466-474.
4. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112, Revised December 2009.
5. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Fourth Edition. CLSI document M29-A4; May 2014.

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**

NeuMoDx Molecular, Inc.
1250 Eisenhower Place
Ann Arbor, MI 48108, USA

Sponsor (AUS):
QIAGEN Pty Ltd
Level 2 Chadstone Place
1341 Dandenong Rd
Chadstone VIC 3148
Australia

EC REP

Emergo Europe B.V.
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

CE 2797

Technical support/Vigilance reporting: support@qiagen.com

Patent: www.neumodx.com/patents