REF **201501 NeuMoDx™ EBV Quant Test Strip 2.0** ℞ 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 EBV Quant Assay 2.0 is an automated *in vitro* nucleic acid amplification test for the quantification of human Epstein-Barr virus (EBV) DNA in EDTA plasma from immunocompromised transplant patients.
The NeuMoDx EBV Quant Assay 2.0 as performed on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System incorporates automated DNA extraction to isolate the target nucleic acids from the specimen and real-time PCR targeting two highly conserved regions in the EBV genome.
The assay is intended for use as an aid in monitoring EBV DNA levels in peripheral blood to assess viral response to treatment. This assay is intended for use in conjunction with clinical presentation and other laboratory markers of disease progression for the clinical management and monitoring of EBV infection.
The assay is not intended for use as a screening test for the presence of EBV DNA in blood or blood products. The NeuMoDx EBV Quant Assay 2.0 is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures and/or NeuMoDx Molecular Systems. The NeuMoDx EBV Quant Assay 2.0 is not intended for self-testing or point-of-care use.

SUMMARY AND EXPLANATION

Human whole blood collected in sterile blood collection tubes containing EDTA as an anticoagulation agent may be used for the preparation of plasma. To initiate testing, plasma in a specimen tube compatible with the NeuMoDx System is placed into a specimen tube carrier and loaded onto the NeuMoDx System worktable. 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 (two highly conserved regions in the EBV genome). The NeuMoDx EBV Quant Assay 2.0 includes a DNA Sample Process Control (SPC1) to help monitor for the presence of potentially inhibitory substances and NeuMoDx System or reagent failures that may be encountered during the extraction and amplification process.
EBV is a common double-stranded DNA virus of the human herpesvirus family that infects people of all ages. It is estimated that > 90% of individuals worldwide are or have been infected with EBV.[1] EBV is spread through body fluids, such as saliva, blood, semen, and organ transplantation. Many people become infected with EBV in childhood. These individuals, while infected with EBV, are typically asymptomatic. Immunocompromised individuals may develop more severe symptoms and complications from EBV infection. Latent EBV infection poses the greatest risk to post-transplant patients. Post-transplant lymphoproliferative disorders (PTLDs) include EBV-driven tumor formation in B cells due to the effect of immunosuppressive agents on the immune control of EBV, one of the most significant causes of morbidity and mortality in patients undergoing any kind of organ transplantation.[2]
EBV viral load monitoring can be used as aid in the diagnosis and management of EBV-associated PTLD. However, diagnosis must be performed with a biopsy. EBV viral load monitoring may also be used to monitor response to EBV-associated PTLD treatment, usually with Rituximab and a reduction in immunosuppressive therapy.[3]

PRINCIPLES OF THE PROCEDURE

The NeuMoDx EBV Quant Assay 2.0 on the NeuMoDx System utilizes the NeuMoDx EBV Quant Test Strip 2.0, NeuMoDx EBV Calibrators, NeuMoDx EBV External Controls, NeuMoDx Lysis Buffer 1, and NeuMoDx general use reagents to perform the analysis. The NeuMoDx EBV Quant Assay 2.0 combines automated DNA extraction, amplification, and detection by real-time PCR. Whole blood specimens are collected in EDTA tubes for the preparation of plasma. The plasma specimen, in a NeuMoDx System compatible specimen tube, is placed into a specimen tube carrier and loaded onto the NeuMoDx System worktable 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 magnetic affinity microspheres. The microspheres, 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 EBV-specific targets and SPC1. 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 NeuMoDx EBV Quant Assay 2.0 targets two highly conserved regions, BALF5 and BXFL1, in EBV genome. The dual target design reduces the risk of false-negative results in the event of mutations in one target region, thus increasing the robustness of the assay. 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 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 causes loss of proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing fluorescence detection of the fluorophore. The resulting fluorescent signal detected 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 and emission: 521 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect both EBV DNA targets. For detection of the SPC1, the TaqMan probe is labeled with an alternate fluorescent dye (excitation: 535 nm and 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/NO RESULT/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 outside of the linear range.

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
201501	**NeuMoDx EBV Quant Test Strip 2.0** *Dried RT-PCR reagents containing EBV and SPC1 specific TaqMan probe and primers.*	6	16	96

Materials Required but Not Provided (Available Separately from QIAGEN)

REF	Contents
800501	**NeuMoDx EBV Calibrators** *Single-use sets of EBV High and Low Calibrators to establish validity of standard curve (1 vial of each control = 1 set)*
900502	**NeuMoDx EBV External Controls** *Single-use sets of EBV Low Positive, High Positive, and Negative Controls to establish daily validity of NeuMoDx EBV Quant Assay 2.0 (1 vial of each control = 1 set)*
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
400500	**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]
NeuMoDx System Software version 1.9.2.6 or higher

WARNINGS AND PRECAUTIONS

- The NeuMoDx EBV Quant Test Strip 2.0 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 NeuMoDx EBV Calibrators [REF 800501]) must be available before test results can be generated for clinical samples.
- NeuMoDx EBV External Controls [REF 900502] must be processed every 24 hours when testing with the NeuMoDx EBV Quant Assay 2.0.
- Minimum specimen volume of secondary aliquots of EDTA plasma is detailed below in Test Preparation section. 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 (DNase) contamination of all reagents and consumables. The use of sterile, DNase-free, disposable transfer 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 EBV Quant Test Strip 2.0, 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 EBV Quant Test Strip 2.0 and the NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer container; 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*[4] and in CLSI Document M29-A4.[5]
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDS).
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations. Follow recommendations in the Safety Data Sheet (SDS).

NeuMoDx EBV Quant Test Strip 2.0

Contains: boric acid. Danger! Causes serious eye irritation. May damage fertility or the unborn child. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF exposed or concerned: Get medical advice/ attention. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Emergency information

CHEMTREC
Outside USA & Canada +1 703-527-3887

PRODUCT STORAGE, HANDLING AND STABILITY

1. NeuMoDx EBV Quant Test Strips 2.0 are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 15°C to 28°C.
2. Once loaded, the NeuMoDx EBV Quant Test Strip 2.0 may remain onboard the NeuMoDx System for 14 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the System.
3. Although noninfectious, NeuMoDx EBV Calibrators and NeuMoDx EBV External Controls should be discarded in laboratory biohazard waste after use to reduce risk of contamination.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Handle all specimens as if they are capable of transmitting infectious agents.

1. Do not freeze whole blood or any specimens stored in primary tubes.
2. To prepare plasma specimens, whole blood should be collected in sterile tubes using EDTA as the anticoagulant. Follow the specimen collection tube manufacturer instructions.
3. 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.
4. 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.
5. 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.
6. Prepared plasma specimens may be stored at -20 °C for up to 8 weeks; plasma samples should not be subjected to more than 2 freeze/thaw cycles prior to use.
    1. If specimens are frozen, allow the specimens to completely thaw at room temperature (15°C to 30°C); vortex to generate a uniformly distributed sample. Samples should be at room temperature prior to testing.
    2. Once frozen samples are thawed, testing should occur within 8 hours.
7. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.

INSTRUCTIONS FOR USE

Test Preparation

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System as described below.
2. Transfer an aliquot of the specimen to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:
3. *For plasma specimens:*
   - Specimen Tube 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 ≥ 1100 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 650 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 EBV Quant Test Strip(s) 2.0 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 and the NeuMoDx Release Reagent and 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 the Calibrators [REF 800501] and/or External Controls [REF 900502] as required. Further information regarding calibrators and controls can be found in the *Results Processing* section.
5. Load the specimen tube(s) into a Specimen Tube Carrier and ensure caps and any swabs 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 test(s) identified, given a valid test order is present in the system.

LIMITATIONS

1. The NeuMoDx EBV Quant Test Strip 2.0 can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx EBV Quant Test Strip 2.0 has been established for plasma specimens prepared from whole blood collected with EDTA as an anticoagulant. The use of the NeuMoDx EBV Quant Test Strip 2.0 with other sources has not been assessed, and performance characteristics are unknown for other specimen types.
3. Because detection of EBV is generally dependent on the number of viral particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
4. 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 EBV Quant Assay 2.0.
5. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
6. If both EBV targets and the SPC1 target do not amplify, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
7. If a system error occurs prior to completion of sample processing, "No Result" will be reported and the test should be repeated.
8. In the event EBV DNA is detected above ULoQ, the NeuMoDx EBV Quant Assay 2.0 may be repeated with a diluted aliquot of the original specimen. A 1:100 or 1:1000 dilution in EBV negative plasma or Basematrix 53 Diluent (Basematrix, SeraCare®, Milford, MA) is recommended. The System will automatically calculate the concentration of the original specimen as follows: Original specimen concentration = log10 (dilution factor) + reported concentration of the diluted sample, as long as the dilution factor has been properly selected in the software before repeating.
9. The 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.
10. A positive result is indicative of the presence of EBV DNA.
11. Although the possibility is low, deletions or mutations in the conserved regions targeted by the NeuMoDx EBV Quant Assay may affect detection and/or quantification and could lead to an erroneous result.
12. Results from NeuMoDx EBV Quant Assay 2.0 should be used as an adjunct to clinical observations and other information available to the physician; the test is not intended to diagnose infection.
13. 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 EBV Quant Assay 2.0 results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx EBV Quant Assay Definition File (EBV Quant ADF version 4.0.0 or higher). A NeuMoDx EBV Quant Assay 2.0 result may be reported as Negative, Positive with a reported EBV DNA concentration, Indeterminate, No Result, or Unresolved based on the amplification status of the target and sample process control. Results are reported based on the ADF results processing decision algorithm, summarized below in *Table 1*.

Table 1: NeuMoDx EBV Quant Assay 2.0 Results Interpretation

Result	EBV Targets	Sample Process Control (SPC1)
Positive	**AMPLIFIED** [2 ≤ Ct < 28 AND EPR > 1.3 AND EP > 1200] OR [28 < Ct < 38 AND EP > 1200]	N/A
Positive, above Upper Limit of Quantitation [ULoQ] (Log10 IU/mL)	[CONC] > 8.0 Log10 IU/mL, NO QUANT	N/A
Positive, below Lower Limit of Quantitation [LLoQ] (Log10 IU/mL)	[CONC] < 1.48 Log10 IU/mL, NO QUANT	N/A
Negative	**NOT AMPLIFIED** N/A **OR** [2 ≤ Ct < 28 AND EPR ≤ 1.3 AND EP > 1200] **OR** [28 ≤ Ct < 38 AND EP > 1200] **OR** Ct > 38	**AMPLIFIED** [29 < Ct < 35 and EP ≥ 2000]
No Result*	Not Amplified; System Error Detected; Sample Processing Aborted	
Indeterminate*	Not Amplified; System Error Detected; Sample Processing Completed	
Unresolved*	Not Amplified, No System Error Detected	

EP = End Point Fluorescence; EPR = End Point Fluorescence Ratio; C_t = Cycling Threshold;
Quant = calculated quantity of EBV present expressed in $\log_{10}$ IU/mL. See Test Calculation section below.
* The System allows optional Rerun/Repeat capability to enable automatic reprocessing in the event of an invalid result to minimize delays in result reporting.

Test calculation: Samples

1. For samples within the linear range of the NeuMoDx EBV Quant Assay 2.0, the concentration of EBV DNA in the samples is calculated using the stored standard curve in conjunction with the calibration coefficient.
    1. A "calibration coefficient" is calculated based on the results of the NeuMoDx EBV Calibrators processed to establish validity of the Standard Curve for each lot of the NeuMoDx EBV Quant Test Strip 2.0 on a specific NeuMoDx System.
    2. The calibration coefficient is incorporated automatically by the System into the final determination of the concentration of EBV DNA.
2. NeuMoDx EBV Quant Assay 2.0 results are reported in IU/mL and Log10 IU/mL.
3. The resulting quantitation of the unknown samples is traceable to the 1st WHO International Standard for Epstein-Barr virus for Nucleic Acid Amplification Techniques.

Test Calculation: Calibrators

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

1. NeuMoDx EBV Calibrators are provided in a kit [REF 800501] and contain non-infectious encapsulated EBV target prepared in Basematrix.
2. A set of EBV Calibrators needs to be processed with each new lot of NeuMoDx EBV Quant Test Strip 2.0 if a new EBV Assay Definition File is uploaded to the NeuMoDx System, if the current set of calibrators are past the validity period (set at 90 days), or if the NeuMoDx System software is modified.
3. The NeuMoDx System software will notify the user 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:
    1. A set of two calibrators – high and low – need to be processed to establish validity.
    2. 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 Log10 IU/mL and the High Calibrator nominal target is 5 Log10 IU/mL.
    3. A calibration coefficient is calculated to account for expected variation between test strip lots; this calibration coefficient is utilized in determination of the final EBV DNA concentration.
5. If one or both calibrators fail the validity check, repeat the 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 the 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 QIAGEN Technical Support.

Invalid Results

If a NeuMoDx EBV Quant Assay 2.0 performed on the NeuMoDx System fails to produce a valid result, it will be reported as either Indeterminate, No Result, or Unresolved; based on the type of error that occurred, the test should be repeated to obtain a valid result.

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

A No Result will be reported if a NeuMoDx System error is detected and sample processing is aborted. In the event of a No Result, a retest is recommended.

An Unresolved result will be reported if no target is detected and there is no amplification of the Sample Process Control, which indicates possible reagent failure or the presence of inhibitors. In the event of an Unresolved result, a retest is recommended as a first step. If the retest fails, a diluted specimen may be used to mitigate the effect of possible inhibition (see limitations section for further instructions).

See the NeuMoDx 288 Molecular System Operator's Manual (PN: 40600108) or the NeuMoDx 96 Molecular System Operator's User Manual (PN: 40600317) for a list of error codes that may be associated with Invalid Results.

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 controls containing non-infectious encapsulated EBV target in Basematrix for positive controls, or Basematrix for negative controls, are provided by QIAGEN in a kit containing the NeuMoDx EBV External Controls [REF 900502].
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:

NeuMoDx EBV External Controls	Expected Concentration	Label Color Scheme
NeuMoDx EBV High Positive Control (C1EBV)	1.5E4 IU/mL (4.18 Log_{10} IU/mL)	Red
NeuMoDx EBV Low Positive Control (C2EBV)	150 IU/mL (2.18 Log_{10} IU/mL)	Grey
NeuMoDx EBV Negative Control (NCEBV)	N/A	Black

3. When processing External Controls, place the controls in a specimen tube carrier and use the touchscreen to load the carrier into NeuMoDx System from the autoloader shelf. The NeuMoDx System will recognize the barcodes and start processing controls unless reagents or consumables required for testing are not available.
4. Validity of these external controls will be assessed by the NeuMoDx System based on the expected results.

NeuMoDx EBV External Controls	EBV Quant Result	SPC1 Result
NeuMoDx EBV High Positive Control (C1EBV)	EBV POSITIVE [Conc] 3.68 – 4.68 Log_{10} IU/mL	SPC1 Positive
NeuMoDx EBV Low Positive Control (C2EBV)	EBV POSITIVE [Conc] 1.58 – 2.78 Log_{10} IU/mL	SPC1 Positive
NeuMoDx EBV Negative Control (NCEBV)	EBV NEGATIVE	SPC1 Positive

5. Discrepant result handling for external controls should be performed as follows:
    1. A Positive test result reported for a negative control sample may indicate contamination, and the laboratory's quality control procedures need to be examined to find a root cause. Ensure to use separate areas for sample preparation, control handling, and RT-PCR set up. Please refer to *NeuMoDx 288 or 96 Molecular System Operator's Manual* for additional troubleshooting tips.
    2. A Negative result reported for a positive control sample may indicate there is a reagent or instrument related problem.
    3. In either of the above instances, or in the event of a No Result (NR), Unresolved (UNR), or Indeterminant (IND) result, repeat the failed control with freshly thawed vial(s) of the control(s) failing the validity test.
    4. If the Positive external control continues to report a Negative result, contact QIAGEN technical support.
    5. If the Negative external control continues to report a Positive result, attempt to eliminate all sources of potential contamination, including replacing all reagents and repeat the run before contacting QIAGEN technical support.
6. If the External Controls do not provide the expected results, it is required to repeat a set of positive and negative controls. Sample results will not be reported if controls do not give expected results.
7. The NeuMoDx System is equipped with automatic Rerun/Repeat capability that user can choose to use to ensure that an INVALID result is automatically reprocessed to minimize delays in result reporting.

Sample Processing (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 RT-PCR amplification with each sample/control/calibrator. The primers and probe specific for the SPC1 are included in each NeuMoDx EBV Quant Test Strip 2.0. This SPC1 allows the NeuMoDx System to monitor the efficacy of the DNA extraction and RT-PCR amplification processes.

PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY – Limit of Detection

The analytical sensitivity of the NeuMoDx EBV Quant Assay 2.0 was characterized in two sequential stages: 1. Preliminary Limit of Detection (LoD) Assessment (Probit analysis) followed by 2. LoD Confirmation. In part 1, negative specimens and a dilution series of the WHO 1st International Standard in screened EBV negative human plasma were tested to determine the preliminary LoD on the NeuMoDx Systems. The preliminary LoD was defined as the lowest target level detected at a rate of 95% as determined by Probit analysis. In part 2, the preliminary LoD was confirmed by testing a contrived panel at the LoD level. Both stages of the study were performed over 3 days across multiple systems with multiple lots of NeuMoDx reagents. In part 1, a total of 144 replicates at each dilution level were processed. Detection rates are depicted in Table 2.

Table 2: Preliminary LoD Determination of the NeuMoDx EBV Quant Assay 2.0

Target Concentration [IU/mL]	Target Concentration [log_{10} IU/mL]	PLASMA		
		Number of Valid Tests	Number of Positives	Detection Rate
35	1.54	144	139	96.5%
30	1.48	144	140	97.2%
25	1.40	143	133	93.0%
10	1.00	144	97	67.4%
5	0.70	143	75	52.4%
NEG	---	144	0	0.0%

The LoD of the NeuMoDx EBV Quant Assay 2.0 in plasma using the 1st WHO International Standard for EBV was determined to be 29.3 IU/mL (1.47 log_{10} IU/mL) with 95% Confidence Interval (CI) of 24.4 – 37.1 IU/mL, (1.39 - 1.57 log_{10} IU/mL) [**Figure** *1*]. This LoD was subsequently confirmed by hit-rate analysis which is depicted in Table 3.

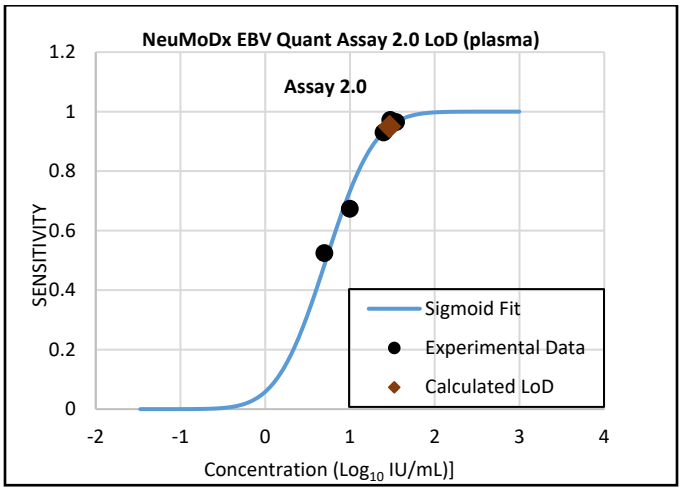

Figure 1: Probit Style Analysis Used to Determine the LoD of the NeuMoDx EBV Quant 2.0 in Plasma Samples

Table 3: LoD Confirmation of the NeuMoDx EBV Quant Assay 2.0

System	Target Concentration [IU/mL]	Target Concentration [log10 IU/mL]	Number of Valid Tests	Number of Positives	Detection Rate
N96	29.3	1.47	96	94	97.9%
N288			96	92	95.8%
All			192	186	96.9%

The LoD for EBV genotype 2 (GT2) was confirmed to be 29.3 IU/mL [1.47 Log10 IU/mL] as determined by hit-rate analysis.

Based on the outcome of both studies, the LoD of the NeuMoDx EBV Quant Assay 2.0 was determined to be 29.3 IU/mL [1.47 Log10 IU/mL].

ANALYTICAL SENSITIVITY –Lower Limit of Quantitation (LLoQ)

The LLoQ is defined as the lowest target level at which >95% detection is achieved AND the total analytical error (TAE) ≤ 1.0. In order to determine the LLoQ, the TAE was calculated for each of the EBV target levels that were shown to report > 95% detection as part of the 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 the 1st WHO International Standard for EBV plasma specimens used in the LLoQ study are shown in *Table 4*. Based on this data set and the previously determined LoD, the LLoQ was determined to be 30.0 IU/mL (1.48 Log_{10} IU/mL) and subsequently confirmed for EBV genotype 2 (GT2).

Table 4: NeuMoDx EBV Quant Assay 2.0 LLoQ, with Bias and TAE

Target Conc. [IU/mL]	Target Conc. [log_{10} IU/mL]	Plasma				
		Average Conc. [log_{10} IU/mL]	Detection Rate	SD	Bias	TAE
35	1.54	2.05	96.5%	0.23	0.50	0.96
30	1.48	1.97	97.2%	0.24	0.49	0.98
25	1.40	1.93	93.0%	0.24	0.53	1.02
10	1.00	1.96	67.4%	0.31	0.96	1.59
5	0.70	1.83	52.4%	0.27	1.13	1.68

Based on the outcome of these studies, the LoD of the NeuMoDx EBV Quant Assay 2.0 was determined to be 29.3 IU/mL (1.47 log_{10} IU/mL) and the LLoQ was determined to be 30.0 IU/mL [1.48 log_{10} IU/mL].

Linearity and Determination of Upper Limit of Quantitation (ULoQ)

Linearity and the Upper Limit of Quantitation (ULoQ) of the NeuMoDx EBV Quant Assay 2.0 were established in plasma by preparing a dilution series using the NeuMoDx encapsulated EBV target and ATCC EBV Culture (ATCC, Manassas, VA) with established traceability to the 1st WHO International Standard for EBV, in addition to 1st WHO International Standard for EBV. A 10-member panel was prepared in pooled EBV negative plasma to create a panel that would span a concentration range of 1.48 – 8.0 Log_{10} IU/mL. The ULoQ of the NeuMoDx EBV Quant Assay 2.0 was determined to be 8.0 Log_{10} IU/mL. A confirmation panel to assess the linearity of the standard curve was prepared, and the EBV assay concentrations reported by the NeuMoDx System compared to the expected values are presented in *Figure 2.*

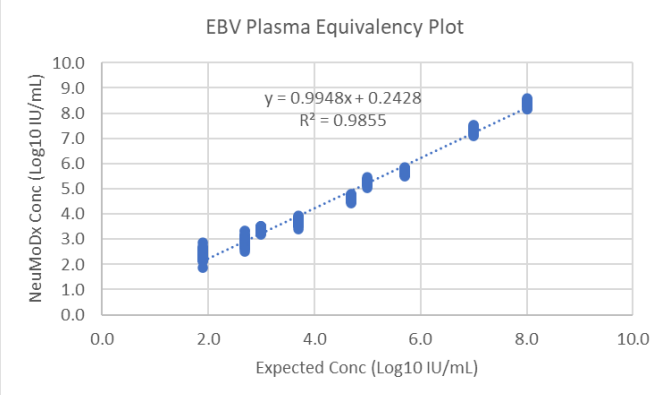

Figure 2: Linearity of the NeuMoDx EBV Quant Assay 2.0

Linearity of EBV Genotype 2 (GT2)

The linearity of the NeuMoDx EBV Quant Assay 2.0 across EBV genotype 2 (GT2) was characterized by testing eleven different concentrations of EBV GT2, with established traceability to the 1st WHO International Standard for EBV, prepared in pooled EBV-negative plasma. The study was performed by testing 36 replicates at 11 concentrations across 2 NeuMoDx Systems and 3 lots of EBV Quant Test Strips 2.0. The linearity for EBV genotype 2 (GT2) is presented in *Table 5* and *Figure 3*.

Table 5: Linearity of the NeuMoDx EBV Quant Assay 2.0 for EBV Genotype 2

Genotype	Linearity Equation y = NeuMoDx EBV Quant Assay 2.0 Quantitation x = Expected Quantitation	R^2
GT2	y = 0.9965x – 0.0982	0.9761

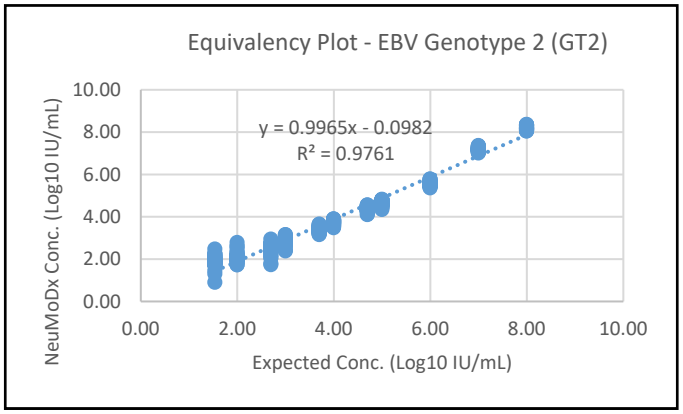

Figure 3: Linearity of the NeuMoDx EBV Quant Assay 2.0 for EBV Genotype 2

Analytical Specificity – Cross-Reactivity

Analytical specificity was demonstrated by screening 36 organisms that may be found in blood/plasma specimens, as well as species phylogenetically similar to EBV for cross-reactivity**.** Organisms at high concentration were prepared in pools of 5-6 organisms. The organisms tested are shown in *Table 6* No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx EBV Quant Assay 2.0.

Table 6: 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*
Cytomegalovirus	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	SV 40	*Mycobacterium avium*	*Staphylococcus epidermidis*	*Chlamydia trachomatis*

Analytical Specificity – Interfering Substances, Commensal Organisms

The NeuMoDx EBV Quant Assay 2.0 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 6*. Negative EBV plasma was spiked with the organisms pooled in groups of 4-7; these pools were then spiked with EBV target at a concentration of 90 IU/mL [1.95 Log_{10} IU/mL]. No significant interference was observed in the presence of these organisms as indicated by minimal deviation of quantitation from control specimens which contained no interfering agent.

Analytical Specificity – Interfering Substances, Endogenous and Exogenous Substances

The NeuMoDx EBV Quant Assay 2.0 was evaluated in the presence of typical exogenous and endogenous interfering substances encountered in EBV clinical plasma specimens. These included abnormally high levels of blood components as well as common antiviral and immunosuppressant medications, classified in *Table 7*. Each substance was added to screened EBV-negative human plasma spiked with EBV at 90 IU/mL [1.95 Log_{10} IU/mL], and samples were analyzed for interference by comparing the reported concentration to the positive control. In addition, common disease state plasma associated with EBV 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 EBV are reported in *Table 8.* None of the exogenous and endogenous substances affected the specificity of the NeuMoDx EBV Quant Assay 2.0.

Table 7: 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 (EBV)		Everolimus	Immunosuppressant
	Valganciclovir hydrochloride	Antiviral (EBV)		Clavulanate potassium	Antibiotic
Pool 2	Prednisone	Corticosteroid/ Immunosuppressant	Pool 5	Famotidine	Histamine receptor antagonist
	Cidofovir	Antiviral (EBV)		Sulfamethoxazole	Antibiotic
	Cefotetan	Antibiotic (broad spectrum)		Valacylovir	Antiviral (Herpesviridae)
	Cefotaxime	Antibiotic (broad spectrum)		Letermovir	Antiviral (EBV)
	Fluconazole	Antifungal		Ticarcillin disodium	Antibiotic
Pool 3	Mycophenolate mofetil	Immunosuppressant		Leflunomide	Immunosuppressant
	Mycophenolate sodium	Immunosuppressant			
	Piperacillin	Antibiotic			
	Sirolimus/ Rapamycin	Immunosuppressant)			
	Tazobactam	Modified antibiotic			

Table 8: Interference Testing – Endogenous and Exogenous Agents

Endogenous + Disease State	Average Conc.	Bias
	Log_{10} IU/mL	Log_{10} IU/mL
Hemoglobin	2.19	0.32
Triglycerides	1.90	0.02
Bilirubin	2.12	0.24
Albumin	1.95	0.07
Systemic Lupus Erythematosus (SLE)	2.08	0.20
Antinuclear Antibody (ANA)	2.36	0.48
Rheumatoid Arthritis (RA)	1.89	0.01
Positive Control	1.88	N/A
Exogenous (Medications)	**Average Conc.**	**Bias**
	Log_{10} IU/mL	Log_{10} IU/mL
Pool 1: Azathioprine, Cyclosporine, Foscarnet, Ganciclovir, Valganciclovir hydrochloride	2.19	0.09
Pool 2: Prednisone, Cidofovir, Cefotetan, Cefotaxime, Fluconazole	2.11	0.01
Pool 3: Mycophenolate mofetil, Mycophenolate sodium, Piperacillin, Sirolimus/Rapamycin, Tazobactam	2.16	0.06
Pool 4: Trimethoprim, Vancomycin, Tacrolimus, Everolimus, Clavulanate potassium	2.24	0.14
Pool 5: Famotidine, Sulfamethoxazole, Letermovir, Valacyclovir, Ticarcillin disodium, Leflunomide	2.26	0.16
Positive Control	2.10	N/A

Within Lab Precision

Precision of the NeuMoDx EBV Quant Assay 2.0 was determined by testing 3 replicates of a 6-member panel of EBV specimens prepared with NeuMoDx EBV Positive Control and EBV culture (ATCC, Manassas, VA) two times per day, using two NeuMoDx 288 Systems and two NeuMoDx 96 System over 12 days. The within-run, within-day, and within-System precisions were characterized, and the overall standard deviation was determined to be $\leqslant$ 0.18 Log_{10} IU/mL. Excellent precision was demonstrated across systems, days, and 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 EBV Quant Assay 2.0 on NeuMoDx Systems

Target EBV Conc. [Log_{10} IU/mL]	Average EBV Conc. [Log_{10} IU/mL]	Within System SD	Within Day SD	Within Run SD	Overall (Within Lab) SD
7.70	7.82	0.10	0.08	0.08	0.11
6.00	6.07	0.12	0.11	0.11	0.13
5.00	4.75	0.13	0.12	0.11	0.13
4.00	3.78	0.13	0.11	0.11	0.14
3.00	2.93	0.15	0.14	0.13	0.16
1.95	2.19	0.17	0.16	0.16	0.18

Lot-to-Lot Reproducibility

Lot to Lot Reproducibility of the NeuMoDx EBV Quant Assay 2.0 was determined by evaluating 3 lots of NeuMoDx EBV Quant Test Strips 2.0 as part of the Within Lab Precision study. A 6-member panel of EBV positive plasma was used to assess performance (*Table 10*). The results generated across lots was analyzed and presented in *Table 10*. The maximum bias was 0.29 Log_{10} IU/mL, and the maximum SD was 0.18 Log_{10} IU/mL for NeuMoDx EBV Quant Assay Test Strips 2.0. Equivalent performance was demonstrated across lots as quantitation of all panel members was within the tolerance specification.

Table 10: Lot to Lot Reproducibility – NeuMoDx EBV Quant Assay 2.0, Test Strip

	Lot 1			Lot 2			Lot 3		
Expected Conc. (Log10 IU/mL)	Avg Conc. (Log10 IU/mL)	Log Conc SD	Abs Bias	Avg Conc. (Log10 IU/mL)	Log Conc SD	Abs Bias	Avg Conc (Log10 IU/mL)	Log Conc SD	Abs Bias
7.70	7.82	0.11	0.12	7.84	0.10	0.14	7.79	0.09	0.09
6.00	6.08	0.12	0.08	6.10	0.10	0.10	6.04	0.10	0.04
5.00	4.77	0.13	0.23	4.78	0.13	0.22	4.71	0.10	0.29
4.00	3.80	0.15	0.20	3.81	0.13	0.19	3.74	0.11	0.26
3.00	2.96	0.16	0.04	2.96	0.15	0.04	2.87	0.16	0.13
1.95	2.20	0.18	0.25	2.22	0.18	0.27	2.16	0.16	0.21

Effectiveness of Sample Process Control

The Sample Process Control (SPC1) is included in the NeuMoDx EBV Quant Assay 2.0 to report process step failures or inhibition affecting performance of the assay. Using the NeuMoDx CMV Quant Assay as a model, the efficacy of SPC1 was tested for plasma specimens under conditions representative of critical processing step failures that could potentially occur during sample processing and that *may not be detected* by the NeuMoDx System performance monitoring sensors. Cytomegalovirus positive specimens (at 3 Log_{10} IU/mL) and negative specimens were challenged under the following conditions: presence of inhibitor, no wash solution delivered, and no wash blow out. Process inefficiencies that had an adverse effect on viral target detection/quantitation were mirrored by performance of SPC1 target as shown in *Table 11*. 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 or viral target detection and quantitation. Therefore, the SPC1 demonstrated success in effectively monitoring assay performance on the NeuMoDx System.

Table 11: Effectiveness of the Sample Process Control for viral DNA in Plasma*

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

*****Cytomegalovirus (CMV) in plasma specimens was used as model system for Sample Process Control Effectiveness assessment.

Cross-contamination

The cross-contamination rate for plasma specimens was determined by processing alternating high positive and negative samples of EBV. Five sets of such checkerboard testing were performed with a total of 60 replicates of EBV-negative plasma and 60 replicates of a spiked EBV plasma at 6.0 Log_{10} IU/mL on both the NeuMoDx 288 and 96 Molecular Systems. Across both system types, all 120 replicates of the negative specimen were reported as negative, demonstrating the occurrence of no cross-contamination during plasma sample processing on the NeuMoDx Systems.

Specimen Matrix Equivalence

Testing was performed to demonstrate equivalency between fresh and frozen plasma specimens using a similar bloodborne virus, CMV, as a model. Fresh specimens were kept at 4 °C until they were spiked with three levels of CMV and tested for equivalency. 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 specimens were compared for equivalency by regression analysis. The data demonstrated excellent equivalency between fresh and frozen plasma specimens with a slope at 1.0 and very low bias (intercept), as presented in *Table 12* below.

Table 12: Specimen Matrix Equivalency

Parameter Requirement	Fresh vs Frozen EDTA
Slope [0.9-1.1]	1.000
Intercept < 0.5 Log_{10} IU/mL	0.020
p-value > 0.05	0.631

Quantitative Performance Characterization

The quantitative performance of the NeuMoDx EBV Quant Assay 2.0 was characterized by processing two commercial EBV Verification Panels from AcroMetrix and Exact Diagnostics (traceable to the 1st WHO International Standard for EBV) on the NeuMoDx Molecular Systems.

Excellent correlation was obtained between the NeuMoDx EBV Quant Assay 2.0 and the two commercial EBV verification panels (*Figure 4*) when analyzed with either the Deming Regression (*Figure 4A*) or Passing-Bablok method (*Figure 4B*).

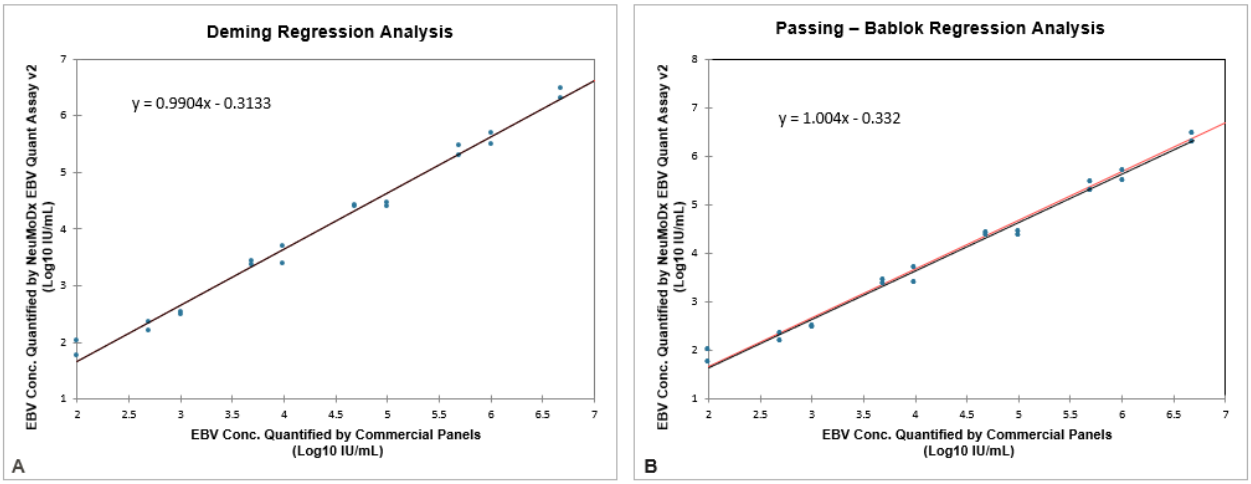

Figure 4. Equivalency Plot Between AcroMetrix and Exact Diagnostics Verification Panels and NeuMoDx EBV Quant Assay. A. Linear regression analysis using Deming method. B. Linear regression analysis using Passing-Bablok method.

The quality of the Deming Regression fit is illustrated by an overall slope coefficient of 0.990 and an intercept (bias) of -0.313, demonstrating that the concentration results obtained between the NeuMoDx EBV Quant Assay 2.0 and the EBV Verification Panels are correlated with acceptable bias. Passing-Bablok linear fit also supports the significance of the correlation between the results obtained from the NeuMoDx EBV Quant Assay 2.0 and EBV Verification Panels with an overall slope coefficient of 1.004 and an intercept (bias) of -0.332. The *p*-value of Passing-Bablok analysis was calculated to be 0.988.

Table 13: Summary of Deming and Passing-Bablok Linear Regression Analysis

Deming Analysis		Passing-Bablok Analysis	
Intercept	Slope Coefficient	Intercept	Slope Coefficient
-0.313 95% CI (-0.620, -0.007)	0.990 95% CI (0.928, 1.053)	-0.332 95%CI (-0.548, -0.116)	1.004 95% CI (0.950, 1.047)

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TRADEMARKS

SYMBOL KEY

℞ only	Prescription use only	Σ	Contains sufficient for *<n>* tests
	Manufacturer		Consult instructions for use
IVD	*In vitro* diagnostic medical device		Caution
EC REP	Authorized representative in the European Community		Health Hazard
REF	Catalog number	CE	CE Mark
LOT	Batch code	CONT	Contains
	Use-by date	BIO	Contains biological material of animal origin
	Temperature limit	Boric Acid	Boric acid
	Do not re-use		

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CE