REF **201500 NeuMoDx™ EBV 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 EBV Quant Assay is an automated, *in vitro* nucleic acid amplification test for the quantitation of human Epstein-Barr virus (EBV) DNA in plasma. The NeuMoDx EBV 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 plasma and real-time polymerase chain reaction (PCR) targeting two highly conserved regions in the Epstein-Barr virus genome.

The NeuMoDx EBV Quant Assay is intended for *in vitro* detection and quantitation of Epstein-Barr virus DNA in fresh and frozen human plasma specimens using NeuMoDx 288 and NeuMoDx 96 Molecular Systems. The NeuMoDx EBV Assay is intended for use in the diagnosis and monitoring of EBV infections. The assay can be used to measure EBV DNA levels to assess response to antiviral treatment. 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 EBV infection. The assay is not intended for use as a screening test for the presence of EBV in blood or blood products.

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 250 µL aliquot of the plasma sample is mixed with NeuMoDx Lysis Buffer 5 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 EBV genome). The NeuMoDx EBV Quant Assay 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 transplantations. Many people become infected with EBV in childhood. These individuals, while infected with EBV, are typically asymptomatic. Immunocompromised people 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]

The use of EBV viral load monitoring facilitates the diagnosis and management of EBV-associated PTLD. However, detection of EBV nucleic acid in blood is not sufficient for the diagnosis of EBV-associated PTLD. Nucleic acid testing (NAT) should only be used in conjunction with clinical presentation and other laboratory markers of disease progression for the clinical management and monitoring of EBV-infected patients. While current guidelines for the management and treatment of EBV 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.[3,4]

PRINCIPLES OF THE PROCEDURE

The NeuMoDx EBV Quant Assay on the NeuMoDx System utilizes the NeuMoDx EBV Quant Test Strip, NeuMoDx EBV Calibrators, NeuMoDx EBV External Controls, NeuMoDx Lysis Buffer 5 and NeuMoDx general use reagents to perform the analysis. The NeuMoDx EBV Quant Assay 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 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 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 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 targets two highly conserved regions, BALF5 and BXFL1, in EBV genome. The dual target design reduces the risk of false negatives in the event of mutation, 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 FRET (Förster Resonance Energy Transfer).

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 (490/521 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect EBV DNA. For detection of the SPC1, the TaqMan probe is labeled with an alternate fluorescent dye (535/556 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software analyzes the data and reports a result (POSITIVE/NEGATIVE/INDETERMINATE/UNRESOLVED). If a result is POSITIVE, the NeuMoDx System software also provides a quantitative value associated with the sample or reports if the calculated concentration is outside of the limits of quantitation.

REAGENTS/CONSUMABLES

Material Provided

REF	Contents	Tests per unit	Tests per package
201500	**NeuMoDx EBV Quant Test Strip** *Dried PCR reagents containing EBV and SPC1 specific TaqMan probe and primers.*	16	96

Additional Materials Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
800500	**NeuMoDx EBV Calibrators** *Single use sets of EBV High and Low Calibrators to establish validity of standard curve*
900501	**NeuMoDx EBV External Controls** *Single use sets of EBV Positive and Negative Controls to establish daily validity of NeuMoDx EBV Quant Assay*
400900	**NeuMoDx Lysis Buffer 5**
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 EBV Quant Assay is for *in vitro* diagnostic use with NeuMoDx Systems only.
- Specimens should always be handled as if they are infectious and in accordance with safe laboratory procedures such as those described in Biosafety in Microbiological and Biomedical Laboratories[5] and in CLSI Document M29-A4.[6]
- A positive result is indicative of the presence of EBV DNA.
- Performance of the NeuMoDx EBV Quant Assay is limited to use by personnel trained on the use of the NeuMoDx System and in the handling of infectious materials.
- 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 800500]) must be available before test results can be generated for clinical samples.

- NeuMoDx EBV External Controls [REF 900501] must be processed every 24 hours throughout testing with the NeuMoDx EBV Quant Assay.
- Minimum specimen volume of secondary aliquots is dependent on the tube size/specimen tube carrier as defined below. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
- The use of specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results.
- Always avoid microbial and deoxyribonuclease (DNase) contamination of all reagents and consumables. The use of sterile DNase-free disposable transfer 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 EBV 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 EBV Quant Test Strip or NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 5 container; 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.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

PRODUCT STORAGE, HANDLING & STABILITY

- NeuMoDx EBV Quant Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 18 to 23 °C.
- Do not use consumables and reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- Do not reload any test product that has previously been loaded onto another NeuMoDx System.
- Once loaded, the NeuMoDx EBV Quant Test Strip 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.
- Although noninfectious, NeuMoDx EBV Calibrators and NeuMoDx EBV External Controls but should be discarded in laboratory biohazard waste after use in laboratory biohazard waste to reduce risk of contamination by the target nucleic acid contained.

SPECIMEN COLLECTION, TRANSPORT & STORAGE

Handle all specimens as if capable of transmitting infectious agents.

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

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

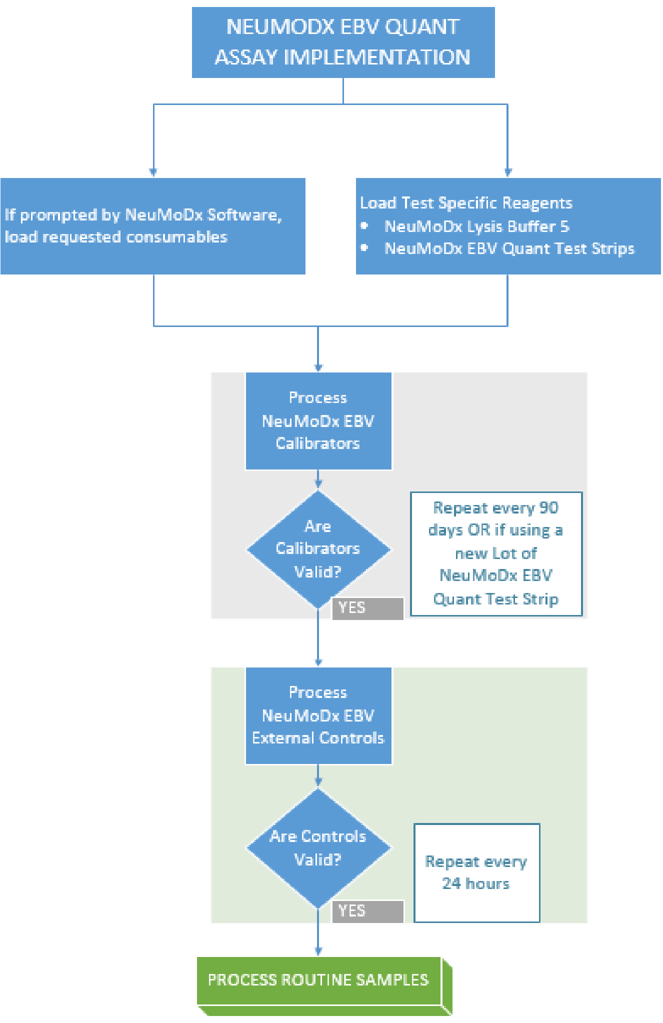


Figure 1: NeuMoDx EBV Quant Assay Implementation Workflow

INSTRUCTIONS FOR USE

Test Preparation

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System.
2. Transfer an aliquot of the plasma to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:
   - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 400 mL
   - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 850 mL

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) 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 Bin as appropriate.
4. If prompted by the NeuMoDx System software, process the Calibrators [REF 800500] and/or External Controls [REF 900501] 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 EBV Quant Test Strip can only be used on NeuMoDx Systems.
- The performance of the NeuMoDx EBV Quant Test Strip has been established for plasma specimens prepared from whole blood collected with EDTA as anticoagulant. The use of the NeuMoDx EBV 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 EBV is dependent on the number of viruses 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 misidentification. 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.
- Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
- If both the EBV targets 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 EBV Quant Assay result is Positive, but the quantitation value is beyond the limits of quantitation, the NeuMoDx System will report whether the detected EBV was *below* Lower Limit of Quantitation (LLoQ) or *above* Upper Limit of Quantitation (ULoQ).
- In the event the detected EBV was below LLoQ, the NeuMoDx EBV Quant Assay may be repeated (if desired) with another aliquot of the specimen.
- In the event the detected EBV is above ULoQ, the NeuMoDx EBV Quant Assay 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.
- 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 active viral infection. Rather, a positive result is presumptive for the presence of Epstein-Barr Virus DNA.
- Although the possibility is very low, deletion or mutations in both the conserved regions of EBV genome targeted by the NeuMoDx EBV Quant Assay may affect detection or could lead to an erroneous result using the NeuMoDx EBV Quant Test Strip.
- Results from NeuMoDx EBV 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 EBV Quant Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx EBV Assay Definition File (EBV ADF). A NeuMoDx EBV Quant Assay result may be reported as Negative, Positive with a reported EBV 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 EBV Quant Assay Decision Algorithm

Result	EBV	Sample Process Control (SPC1)
Positive	[2 $\le$ Ct $\le$ 9 AND EPR > 2 AND EP $\ge$ 1500] OR [9 $\le$ Ct $\le$ 38 AND EP $\ge$ 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] < 2.3 Log_{10} IU/mL, NO QUANT	N/A
Negative	N/A OR [2 $\le$ Ct <9 AND EPR ≤ 2] OR [9 $\le$ Ct $\le$ 38 AND EP < 1500] OR Ct > 38	AMPLIFIED (29 $\le$ Ct $\le$ 35) 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 EBV present expressed in Log_{10} IU/mL. See Test Calculation below.

Test Calculation

1. For samples within the Quantitation range of the NeuMoDx EBV Quant Assay, the concentration of EBV 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 EBV Calibrators processed to establish validity of the Standard Curve, for each lot of the NeuMoDx EBV Quant Test Strips, on a specific NeuMoDx System.
   b. The calibration coefficient is incorporated automatically by the System into the final determination of the concentration of EBV DNA.
2. NeuMoDx EBV Quant Assay results are reported in Log_{10} 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 Calibration

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.

Calibrators

1. NeuMoDx EBV Calibrators are provided in a kit [REF 800500] 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 Strips, 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:
   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 4 Log_{10} IU/mL and the High Calibrator nominal target is 6 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 the final EBV 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 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 EBV target in Basematrix for positive controls, are provided by NeuMoDx Molecular, Inc. in a kit containing the NeuMoDx EBV External Controls [REF 900501].
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 a set of external controls from the freezer and allow the vials to thaw at room temperature (15-30 °C). 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 an EBV Positive result and the negative control should provide an EBV 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 EBV external control(s) with a freshly thawed vial of the control(s) failing the validity test.
   d) If positive NeuMoDx EBV external control continues to report a Negative result, contact NeuMoDx customer service.
   e) If negative NeuMoDx EBV external control continues to report a Positive result, attempt to eliminate all sources of potential contamination, including replacing ALL reagents and consumables 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 EBV Quant Test Strip enabling detection of presence of SPC1 along with the target EBV 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.

If a NeuMoDx EBV 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 EBV 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 EBV Quant Assay was confirmed by testing EBV-negative plasma specimens spiked with a low dilution of the 1st WHO International Standard for EBV for Nucleic Acid Amplification Techniques. This confirmation test was performed at the expected Limit of Detection (LoD) of the NeuMoDx EBV Quant Assay on the NeuMoDx Systems at 200 IU/mL. The LoD was defined as the lowest target level to be detected at a rate of $\geq$ 95%. The study was performed across multiple systems with qualified lots of NeuMoDx reagents. Detection rates are depicted in *Table 2*.

Table 2: NeuMoDx EBV Quant Assay LoD Determination; Positive Detection Rate for Plasma Specimens

Target Concentration [IU/mL]	PLASMA		
	Number of Valid Tests	Number of Positives	Detection Rate
200	120	117	97.5%
0	60	0	0%

Analytical Sensitivity – 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 total analytical error (TAE) ≤ 1.0. In order to confirm that 200 IU/mL as both the LoD and the LLoQ for the EBV Quant Assay, the hit-rate study reults were used to determine the TAE. This calculated TAE was defined:

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.

Table 3: NeuMoDx EBV 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
200	2.30	2.35	97.5	0.28	0.05	0.61

Based on the outcome of these studies, the LoD and LLoQ of the NeuMoDx EBV Quant Assay were both determined to be 200.0 IU/mL [2.30 Log_{10} IU/mL].

Linearity and Determination of Upper Limit of Quantitation (ULoQ)

Linearity and the Upper Limit of Quantitation (ULoQ) of the NeuMoDx EBV Quant Assay were established in plasma by preparing a dilution series using the NeuMoDx encapsulated EBV target and Exact EBV Positive Control (Exact Diagnostics, Fort Worth, TX) with established traceability to the 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 2.0 – 8.0 Log_{10} IU/mL. The ULoQ of the NeuMoDx EBV Quant Assay 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.*

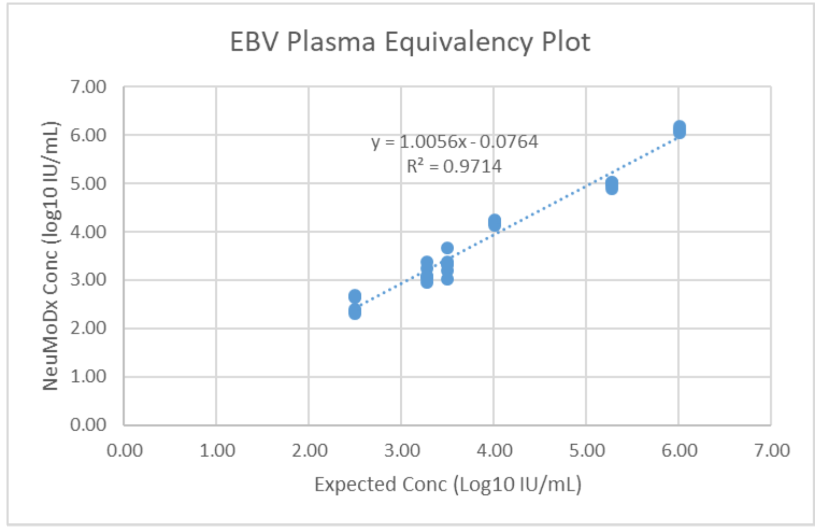


Figure 1: Linearity of the NeuMoDx EBV Quant Assay

Analytical Specificity – Cross-Reactivity

Analytical specificity was demonstrated by screening 35 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 4*. No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx EBV Quant Assay.

Table 4: 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	*Chlamydia trachomatis*	*Mycobacterium avium*	*Staphylococcus epidermidis*	

Analytical Specificity – Interfering Substances, Commensal Organisms

The NeuMoDx EBV 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 4*. 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 3 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 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 5*. Each substance was added to screened EBV-negative human plasma spiked with 3 Log_{10} IU/mL EBV and samples were analyzed for interference. 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 6.* None of the exogenous and endogenous substances affected the specificity of the NeuMoDx EBV Quant Assay.

Table 5: 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 6: Interference Testing - Exogenous and Endogenous Agents

Endogenous	Average Conc.	Bias
	Log_{10} IU/mL	Log_{10} IU/mL
Hemoglobin	3.20	0.23
Triglycerides	3.15	0.28
Bilirubin	3.48	-0.05
Albumin	3.2	0.22
Exogenous (Medications)	**Average Conc.**	**Bias**
	Log_{10} IU/mL	**Log_{10} IU/mL**
Pool 1: Azathioprine, Cyclosporine, Foscarnet, Ganciclovir, Valganciclovir hydrochloride	3.30	0.13
Pool 2: Prednisone, Cidofovir, Cefotetan, Cefotaxime, Fluconazole	3.22	0.21
Pool 3: Mycophenolate mofetil, Mycophenolate sodium, Piperacillin, Sirolimus/Rapamycin, Tazobactam	3.36	0.07
Pool 4: Trimethoprim, Vancomycin, Tacrolimus, Everolimus, Clavulanate potassium	3.32	0.11
Pool 5: Famotidine, Sulfamethoxazole, Letermovir, Valacyclovir, Ticarcillin disodium, Leflunomide	3.47	-0.10
Disease State	**Average Conc.**	**Bias**
	Log_{10} IU/mL	**Log_{10} IU/mL**
Systemic Lupus Erythematosus (SLE)	3.23	0.20
Antinuclear Antibody (ANA)	3.33	0.10
Rheumatoid Arthritis (RA)	3.19	0.24

Within Lab Precision

Precision of the NeuMoDx EBV Quant Assay was determined by testing 3 replicates of a 4-member panel of EBV specimens prepared with EBV Positive Control (Exact Diagnostics, Fort Worth, TX) three times per day, using two NeuMoDx 288 Systems and one NeuMoDx 96 System over two days. The within-run, within-day and within-System precisions were characterized, and the overall standard deviation was determined to be ≤ 0.33 Log_{10} IU/mL. Excellent precision was demonstrated across systems, days, and runs as shown in *Table 7*. Precision between operators was not characterized, as the operator plays no significant role in the processing of samples using the NeuMoDx System.

Table 7: Within Lab Precision – NeuMoDx EBV Quant Assay 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
5.2	5.30	0.27	0.25	0.25	0.27
4.2	4.25	0.21	0.21	0.12	0.21
3.2	3.38	0.22	0.20	0.20	0.22
2.7	3.03	0.30	0.30	0.30	0.33

Lot to Lot Reproducibility

Lot to Lot Reproducibility of the NeuMoDx EBV Quant Assay was determined by evaluating three lots of key reagents – NeuMoDx EBV Quant Test Strips and Lysis Buffer 5 – as part of Qualification Testing (QT). A 4-member panel of EBV positive plasma was used to assess performance (*Table 8*). The variation within and across lots was analyzed and results presented in *Tables 8-9*. The maximum overall bias was 0.03 Log_{10} IU/mL and maximum overall SD was 0.20 Log_{10} IU/mL for NeuMoDx EBV Quant Assay Test Strips. The maximum overall bias was 0.12 Log_{10} IU/mL and maximum overall SD was 0.41 Log_{10} IU/mL for NeuMoDx Lysis Buffer 5. Equivalent performance was demonstrated across lots as quantitation of all panel members was within the tolerance specification.

Table 8: Lot to Lot Reproducibility – NeuMoDx EBV Quant Assay, Test Strip

Target EBV Conc. [IU/mL]	Average EBV Conc. [Log_{10} IU/mL]	N (Valid Results Per Lot)	Bias	Between Lot SD	Within Lot SD	Overall SD
5.0	4.98	18	0.02	0.06	0.08	0.10
4.0	3.98	18	0.02	0.08	0.09	0.12
3.0	3.02	18	0.02	0.06	0.10	0.12
2.0	2.03	18	0.03	0.05	0.20	0.20

Table 9: Lot to Lot Reproducibility – NeuMoDx EBV Quant Assay, Lysis Buffer 5

Target EBV Conc. [Log_{10} IU/mL]	Average EBV Conc. [Log_{10} IU/mL]	N (Valid Results Per Lot)	Bias	Between Lot SD	Within Lot SD	Overall SD
5.0	4.97	5	0.03	0.05	0.03	0.06
4.0	3.96	5	0.04	0.22	0.10	0.24
3.0	3.03	5	0.03	0.09	0.11	0.15
2.0	2.12	5	0.12	0.39	0.13	0.41

Effectiveness of Sample Process Control

The Sample Process Control (SPC1) is included in the NeuMoDx EBV Quant Assay 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 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 or viral target 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 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 a similar bloodborne DNA virus, Cytomegalovirus (CMV). Three sets of such checkerboard testing were performed with a total of 108 replicates of CMV-negative plasma and 108 replicates of a spiked CMV plasma at 6.0 Log_{10} IU/mL. All 108 replicates of the negative specimen were reported as negative, demonstrating the occurrence of no cross-contamination during plasma sample processing on the NeuMoDx System.

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. 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 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 11* below.

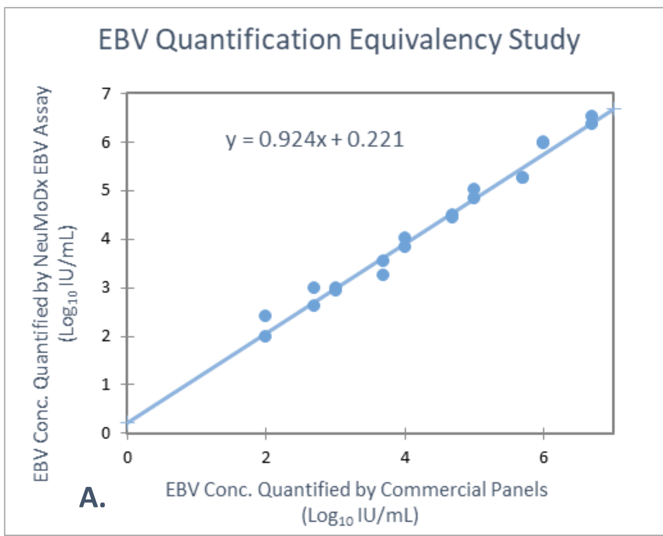
Table 11: Specimen Matrix Equivalency

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

Quantitation Performance Characterization

The quantitative performance of the NeuMoDx EBV Quant Assay was were 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 and the two commercial EBV verification panels (*Figure 4*) when analyzed with either the Deming Regression (*Figure 4A*) or Passing-Bablok method (*Figure 4B*).



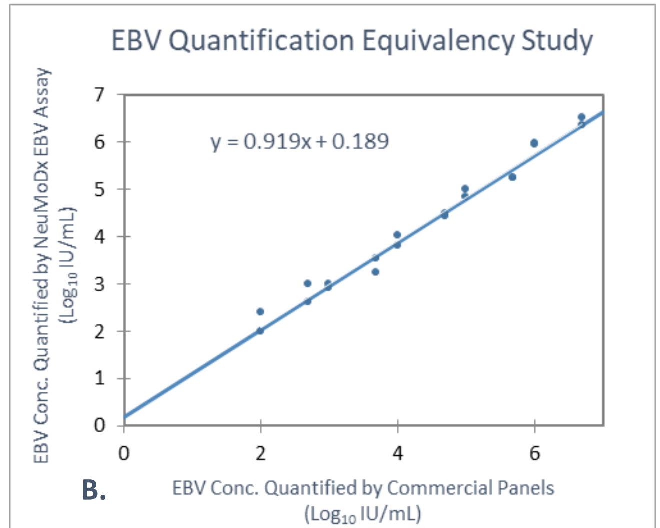


Figure 4. Equivalency Plot Between AcroMetrix and Exact Diagnostics Verification Panels and NeuMoDx EBV Quant Assay. A. Linear regression analysis using Deming method. B. Linear regression analysis using Passing-Bablok method.

The quality of the Deming Regression fit is illustrated by an overall slope coefficient of 0.92 and an intercept (bias) of 0.22, demonstrating that the concentration results obtained between the NeuMoDx EBV Quant Assay 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 and EBV Verification Panels with an overall slope coefficient of 0.92 and an intercept (bias) of 0.19. The *p*-value of Passing-Bablok analysis was calculated to be 0.40.

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

Deming Analysis		**Passing-Bablok Analysis**	
Intercept	Slope Coefficient	Intercept	Slope Coefficient
0.22 95% CI (-0.11, 0.55)	0.92 95% CI (0.86, 0.99)	0.19 95%CI (-0.08, 0.41)	0.92 95% CI (0.87, 0.99)

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SYMBOLS

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

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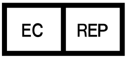

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