QUANTITATIVE DETECTION OF EPSTEIN-BARR VIRUS (EBV) IN PLASMA AND WHOLE BLOOD

Lijie Gong*, Maureen Carey, Kristin Krasch, Jessica Zhu, Michelle Mastronardi, Betty Wu, Sundu Brahmasandra, NeuMoDx Molecular, Ann Arbor, MI

BACKGROUND

Immuno-compromised individuals lacking EBV antibodies, are especially at risk for acute EBV infection that may cause significant mortality and morbidity in organ transplant recipients because of posttransplant lymphoproliferative disorders (PTLD). EBV DNA can be detected in the blood of infected patients and increasing levels of EBV DNA has been shown to correlate significantly with subsequent development of PTLD in susceptible patients. As a result, EBV viral load is important tool for monitoring patients at risk for PTLD and provides prognostic information for effective treatment and management of such patients. The NeuMoDx EBV Test is a "sample to result" type *in-vitro* diagnostic assay incorporating automated extraction of qPCR ready DNA from whole blood and plasma specimens, combined with a sensitive real-time PCR assay to deliver highly accurate results in a completely automated manner.

METHODS

Performance of the NeuMoDx EBV Test was characterized in both plasma and whole blood matrices. The objective of this study was to test and report performance of the NeuMoDx EBV Test across key analytical performance metrics. Studies were performed to characterize the analytical sensitivity, limits of quantitation, linearity, precision, turnaround time, and equivalency across specimen types. Testing was performed using either a 550 µL sample of plasma or a 220 µL sample volume of whole blood specimen.

RESULTS

Evaluation of the analytical sensitivity using a Probit style analysis was performed using the 1st WHO International EBV Standard, and the lower limit of quantitation (LLoQ) was determined using the Total Analytic Error <1.0 criterion. In plasma specimens, the NeuMoDx EBV Test was able to demonstrate a limit of detection (LoD) of 18.8 IU/mL and an LLoQ of 20.2 IU/mL. In whole blood specimens, the NeuMoDx EBV Test demonstrated an LoD of 80 IU/mL (hit rate analysis) with an LLoQ of ≤100 IU/mL (TAE <1.0). The NeuMoDx EBV Test demonstrated excellent linearity across typical, clinically relevant measurement dynamic ranges (R2 ~0.99 across 8 logs), as well as precision across systems, days, and reagent lots. Turnaround time (TAT) for the NeuMoDx EBV Test was ~62 min and the results interpretation module incorporated for automated processing of data provided extremely accurate results. No cross-reactivity was observed against any of the non-target pathogens tested and the test performed efficaciously in the presence of interfering moieties. Finally equivalent performance was demonstrated across both plasma and whole blood specimen matrices.

EBV Analytical Sensitivity (LoD & LLoQ)

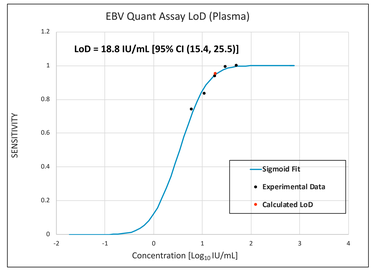
PLASMA

The Limit of Detection of the NeuMoDx EBV Test was determined with pooled EBV negative plasma spiked with 1st WHO International EBV Standard at six different levels including negative samples.

The limit of detection of EBV was determined to be 18.8 IU/mL based on Probit style analysis and the calculated LLoQ was determined to be 20.2 IU/mL.

NeuMoDx EBV Test Limit of Detection - Plasma

Target Conc. (IU/mL)	Target Conc (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
50	1.70	108	108	100	18.8 IU/mL 95% CI (15.4-25.5 IU/mL)
30	1.48	107	106	99.1	
18	1.26	108	101	93.5	
11	1.04	108	90	83.3	
6	0.78	108	80	74.1	
0	N/A	108	0	0	



Limit of Detection of the NeuMoDx EBV Test. Probit analysis from the data in the above table was used to determine the LoD of the EBV target to be 18.8 IU/mL with a 95% CI of (15.4, 25.5).

NeuMoDx EBV Test LLoQ - Plasma

Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	Ave. Bias	Standard Deviation	Total Analytical Error (TAE)	LLoQ (IU/mL)
50	1.70	108	0.32	0.16	0.64	20.21 IU/mL or 1.31 Log_{10} IU/mL
30	1.48	107	0.47	0.25	0.96	
18	1.26	108	0.62	0.18	0.97	
11	1.04	108	0.81	0.22	1.25	
6	0.78	108	0.89	0.25	1.39	

Lower limit of quantitation (LLoQ) of NeuMoDx EBV Test. The lowest target level detected at a positive rate > 95% AND with TAE (bias+2*SD) ≤ 1.0 was used to determine the LLoQ. The LLoQ of the NeuMoDx EBV Test was determined to be 20.21 IU/mL.

WHOLE BLOOD

Based on the LoD and LLoQ established in plasma, the Limit of Detection of the NeuMoDx EBV Test in whole blood was tested with EBV negative blood spiked with 1st WHO International EBV Standard by testing a confirmatory level of 80 IU/mL.

The limit of detection of EBV in whole blood was determined to be ≤80 IU/mL based on 100% amplification of target at this level. The calculated LLoQ was determined to be ≤100 IU/mL (TAE <1.0).

EBV Linearity

PLASMA

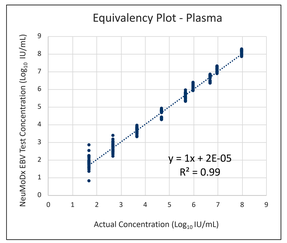
The linearity of the NeuMoDx EBV Test was determined by diluting purified EBV stock (Exact Diagnostics) and NeuMoDx Encapsulated EBV Control in pooled EBV negative plasma to create a panel spanning 8 logs of EBV concentrations ranging from 1.7 Log_{10} IU/mL to 8 Log_{10} IU/mL.

The NeuMoDx EBV Test demonstrated excellent robust quantification linearity over a wide dynamic range (8 log units).

NeuMoDx EBV Test Linearity - Plasma

Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)	Calculated Linear Fit
1.00E+08	8.00	8.06	0.06	8.00
1.00E+07	7.00	7.07	0.07	7.00
1.00E+06	6.00	6.13	0.13	6.00
5.00E+06	6.70	6.60	0.10	6.70
5.00E+05	5.70	5.61	0.09	5.70
5.00E+04	4.70	4.61	0.09	4.70
5.00E+03	3.70	3.63	0.07	3.70
5.00E+02	2.70	2.67	0.03	2.70
5.00E+01	1.70	1.83	0.13	1.70

Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test demonstrated excellent linearity across 8 Log_{10} units.



Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test shows excellent robust correlation over 8 Log_{10} units in plasma.

WHOLE BLOOD

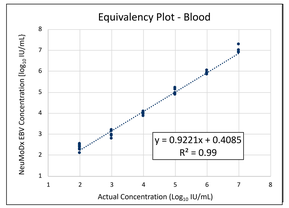
The linearity of the NeuMoDx EBV Test was determined by diluting purified EBV stock (Exact Diagnostics) and 1st WHO International EBV Standard in pooled EBV negative blood to create a panel spanning 6 logs of EBV concentration ranging from 2 Log_{10} IU/mL to 7 Log_{10} IU/mL.

The NeuMoDx EBV Assay demonstrated excellent and robust linearity across 6 log units.

NeuMoDx EBV Test Linearity - Blood

Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)	Calculated Linear Fit
1.00E+07	7.00	7.00	0.00	6.86
1.00E+06	6.00	5.93	0.07	5.94
1.00E+05	5.00	5.02	0.02	5.02
1.00E+04	4.00	3.97	0.03	4.10
1.00E+03	3.00	3.00	0.00	3.17
1.00E+02	2.00	2.36	0.36	2.25

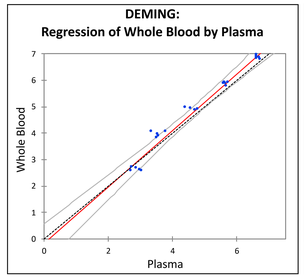
Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test is linear over 6 Log_{10} units.



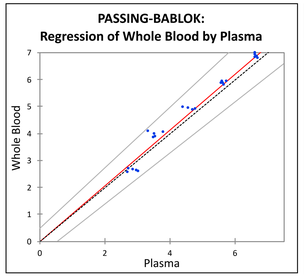
Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test shows excellent correlation over 6 Log_{10} units.

EBV PLASMA TO WHOLE BLOOD EQUIVALENCY

The equivalency of the NeuMoDx EBV Test between matrices, plasma and whole blood was evaluated by spiking K2EDTA plasma and whole blood (screened negative for EBV) with equivalent amount of Exact EBV and 1st WHO International EBV Standard. Levels were spiked to span the linear range of the test. Specimens were then processed on the System. The NeuMoDx EBV Test demonstrated excellent quantitative correlation between plasma and whole blood matrices with Deming and Passing-Bablok linear regression analysis.

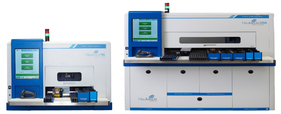


Deming Analysis
Intercept
-0.18 95% CI (-0.66, 0.30)
Slope Coefficient
1.07 95%CI (0.99,1.15)
Correlation Coefficient
R^2=0.99



Deming Analysis
Intercept
-0.004 95% CI (-0.51, 0.48)
Slope Coefficient
1.04 95%CI (0.95,1.13)
p-value
0.25

NeuMoDx Molecular System Streamlined Testing



FEATURES

- **Integrated Operation:** Automates all steps of molecular diagnostics starting from raw clinical specimens to providing real-time PCR results in a fully automated process
- **True Random Access:** Ability to mix specimen types and tests
- **High Throughput:** ~300 DNA tests in an 8 hour shift for the N288, ~150 DNA tests in an 8 hour shift for the N96
- **Fast Time to First Results:** ~60 min
- **Continuous Loading of Specimens:** Specimens and Reagents can be loaded/unloaded at any time
- **Large Walk-Away Window:** Up to 288 samples for the N288, 96 samples for the N96
- **Seamless On Demand Operation:** Automated inventory management of consumables and reagents
- **Long In-Use Shelf Life:** On-board room temperature stable reagents
- **Real-time PCR:** Five-color fluorescence detection offers real-time PCR multiplexing ability

On board storage of room temperature stable reagents enables easy on demand operation.

Patented microfluidic cartridges: capable of performing independent sample processing and real-time PCR.

Analytical Specificity (Cross-Reactivity) & Interference

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

EBV Analytical Specificity – Cross-Reactivity. The analytical specificity of the NeuMoDx EBV Test was evaluated with EBV free plasma spiked with 35 non-target (or phylogenetically similar) organisms (Table above) found in blood/plasma specimens at high concentrations*. No cross-reactivity was observed in any of the organisms listed in the table – demonstrated 100% analytical specificity.

**Bacteria were spiked at ~ 6E6 CFU/mL; Most of the viruses were spiked at 1E6-1E7 IU or copies/mL, except Adenovirus and HIV-2 were spiked at 1E4-1E6 TCID50/mL. DNA was spiked at 1E6 copies/mL.*

NeuMoDx EBV Test Interference - Commensals

Non-Target Organism	Average Conc. (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)
BK Virus, CMV, HHV-6, HHV-7, HHV-8 and HBV	3.11	0.17
Adenovirus 5, HCV, Parvo B19, JC Virus, HPV-16 and HPV-18	3.02	0.08
HSV-1, HSV-2, JC Virus, HIV-1 and HIV-2	3.11	0.17
Chlamydia trachomatis, Clostridium perfringens, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae and Listeria monocytogenes	3.13	0.19
Mycobacterium avium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Propionibacterium acnes, Salmonella typhimurium and Staphylococcus aureus	3.12	0.18
Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Cryptococcus neoformans, Candida albicans and Aspergillus niger	3.13	0.19

Interfering Substances – Commensal Organisms. When NeuMoDx EBV Test was performed with EBV positive plasma prepared by spiking negative plasma with EBV targets at 1E3 IU/mL in the presence of the same 35 non-target organisms listed in TABLE (pooled), minimal deviation of quantitation from EBV control samples were observed indicating no significant interference from any of the 35 organisms.

**Concentration of EBV controls: 3 Log_{10} IU/mL*

NeuMoDx EBV Test Interference - Endogenous Substances

Endogenous	Average Conc. (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)
Albumin (120 mg/mL)	3.11	0.07
Bilirubin (0.02 mg/mL)	3.16	0.02
Hemoglobin (3 mg/mL)	3.12	0.07
Triglycerides (5 mg/mL)	3.21	0.03

EBV Interfering Substances – Endogenous Substances. The possible interference of endogenous substances on NeuMoDx EBV Test was evaluated with four (4) endogenous substance specimens listed in the Table. The NeuMoDx EBV Assay had minimal deviation of quantitation from EBV control samples, with no significant interference.

**Concentration of EBV controls: 3 Log_{10} IU/mL*

Precision and Lot-to-Lot Reproducibility

The Within Lab Precision and Lot-to-lot Reproducibility of the NeuMoDx EBV Test was determined by testing a 4 member panel of EBV on multiple NeuMoDx Molecular Systems across multiple reagent lots and in multiple days.

The same concentrations of EBV were used across multiple lots of reagents and compared to each other for consistency.

The precision Within-run and Across-runs was characterized and the standard deviation for both was determined to be < 0.2 Log_{10} IU/mL. The standard deviation between reagent lots was determined to be ≤ 0.15 Log_{10} IU/mL.

NeuMoDx EBV Test Within Lab Precision

Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	N	Across System SD	Within Run SD	Within Day SD	Overall SD
1	5.70	5.77	84	0.08	0.06	0.02	0.10
2	4.70	4.77	84	0.09	0.07	0.02	0.11
3	3.70	3.77	84	0.11	0.08	0.04	0.13
4	2.70	2.91	80	0.14	0.14	0.05	0.19

NeuMoDx EBV Test Lot-to-lot Reproducibility

Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	N	Between Lot SD	Within Lot SD	Overall SD
1	5.70	5.75	48	0.06	0.06	0.09
2	4.70	4.76	48	0.04	0.08	0.09
3	3.70	3.75	48	0.04	0.07	0.08
4	2.70	2.84	48	0.10	0.11	0.15

Precision and Reproducibility of the NeuMoDx EBV Test. The NeuMoDx EBV Test demonstrated excellent within laboratory precision calculated from the quantitative data across target levels, reagent lots, and Systems with a maximum overall standard deviation < 0.20 Log_{10} IU/mL.

CONCLUSIONS

The NeuMoDx EBV Test demonstrated excellent performance and is well suited for implementing viral load monitoring using both plasma and whole blood specimens.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help and support provided by all members of the NeuMoDx team.

NeuMoDx EBV Test is not available for sale.