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

Lijie Gong[1], Maureen Carey[1]*, Kristin Krach[1], Jessica Zhu[1], Michelle Mastronardi[1], Darren Tomlinson[2], Betty Wu[1], Sundu Brahmasandra[1]
[1]NeuMoDx Molecular, Ann Arbor, MI United States [2]Qiagen, Manchester, United Kingdom

BACKGROUND

EBV-associated post-transplant lymphoproliferative disease (PTLD) often occurs within the first year following transplantation. Increasing levels of EBV DNA in both whole blood and plasma has been shown to correlate significantly with subsequent development of PTLD in susceptible patients. As a result, EBV viral load is an important tool in monitoring transplant recipients to provide prognostic information for effective patient management. 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, coupled 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. The objective of this study was to demonstrate performance of the NeuMoDx EBV Assay across key analytical performance metrics including analytical sensitivity (LoD), linearity, precision, turnaround time, and equivalency across matrices. Evaluation of the analytical sensitivity was performed using the 1st WHO International Standard for EBV, and the limits of quantitation (LLoQ/ULoQ) were determined using the TAE ≤ 1.0 criterion.

RESULTS

The NeuMoDx EBV Test demonstrated a limit of detection (LoD) of 19 IU/mL and a LLoQ of 20.2 IU/mL in plasma and an LoD of 80 IU/mL with an LLoQ of 100 IU/mL in whole blood. The NeuMoDx EBV Test showed excellent linearity across a 7-log dynamic range (R2 > 0.98) with ULoQ of 7 Log10 IU/mL. Excellent quantitative precision across 3 systems over 12 days (maximum deviation of 0.15 Log10 IU/mL), as well as quantitative accuracy across multiple reagent lots was demonstrated. The integrated results interpretation module provided extremely accurate results with a time to first results of only ~60 minutes. No cross-reactivity was observed against any of the 35 non-target pathogens tested, and the test performed efficaciously in the presence of endogenous and exogenous interfering moieties. Finally, equivalent performance was demonstrated across plasma (fresh and frozen) and fresh whole blood specimens.

Within Lab 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 four member panel of EBV on multiple NeuMoDx Molecular Systems across multiple reagent lots on multiple days.

The precision Within-run and Across-runs was characterized and the standard deviation for both was determined to be < 0.2 Log10 IU/mL. The standard deviation between reagent lots was determined to be ≤ 0.15 Log10 IU/mL.

NeuMoDx EBV Test Within Lab Precision							
Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} U/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

Within Lab Precision of the NeuMoDx EBV Test. The NeuMoDx EBV Test demonstrated excellent within laboratory precision calculated from the quantitative data across target levels, Systems and days with a maximum overall standard deviation < 0.20 Log10 IU/mL.

NeuMoDx EBV Test Lot-to-lot Reproducibility						
Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} U/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

Lot-to-Lot Reproducibility of the NeuMoDx EBV Test. The NeuMoDx EBV Test demonstrated excellent reproducibility calculated from the quantitative data across target levels, and across reagent lots with a maximum overall standard deviation ≤ 0.15 Log10 IU/mL.

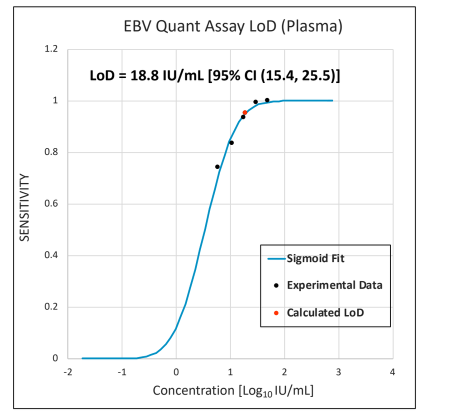
EBV Analytical Sensitivity (LoD & LLoQ)

PLASMA

The Limit of Detection of the NeuMoDx EBV Test was determined with pooled EBV negative plasma spiked with 1st WHO International Standard for EBV 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)
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 extrapolated from the TAE values of the data in the above table 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 Quant Assay in whole blood was tested with EBV negative blood spiked with 1st WHO International Standard for EBV 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

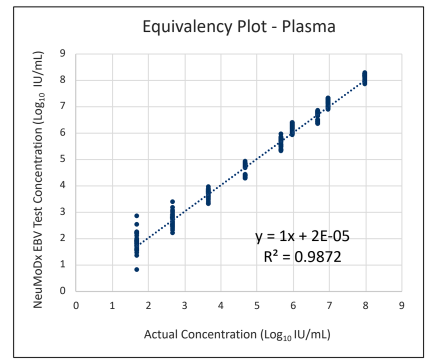
PLASMA

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 Log10 IU/mL to 8 Log10 IU/mL.

The NeuMoDx EBV Test demonstrated excellent and 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)	Calc. 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 in K2EDTA Plasma. The NeuMoDx EBV Test demonstrated excellent linearity across 8 Log10 units.



Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test shows excellent and robust correlation over 8 Log10 units in plasma.

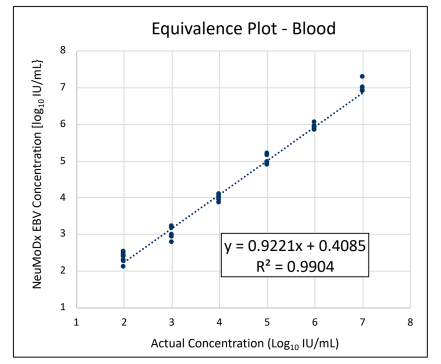
WHOLE BLOOD

The linearity of the NeuMoDx EBV Test was determined by diluting purified EBV stock (Exact Diagnostics) and 1st WHO International Standard for EBV, in EBV-negative blood, to create a panel spanning 6 logs of EBV concentration ranging from 2 Log10 to 7 Log10IU/mL.

The NeuMoDx EBV Assay demonstrated excellent and robust linearity across 6 Log10 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)	Calc. Linear Fit
1.00E+07	7.00	7.00	0.00	6.86
1.00E+06	6.00	5.93	0.07	5.94
1.00+05	5.00	5.02	0.02	5.02
1.00+04	4.00	3.97	0.03	4.10
1.00+03	3.00	3.00	0.00	3.17
1.00+02	2.00	2.36	0.36	2.25

Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test is linear over 6 Log10 units.



Linear range of the NeuMoDx EBV Test. The NeuMoDx EBV Test shows excellent correlation over 6 Log10 units.

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:** 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 & Interference

NeuMoDx EBV Quant Assay-Analytical Specificity					
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 phylogenetically similar or commensal organisms found in blood/plasma specimens at high concentrations* (Table above). No cross-reactivity was observed in any of the organisms listed in the above table; demonstrating 100% analytical specificity.

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

NeuMoDx EBV Quant Assay Interference - Commensals		
Non-Target Organisms	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. 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 tested for cross-reactivity. Organisms were tested in pools. Minimal deviation of quantitation from EBV control samples was observed indicating no significant interference from any of the 35 organisms.

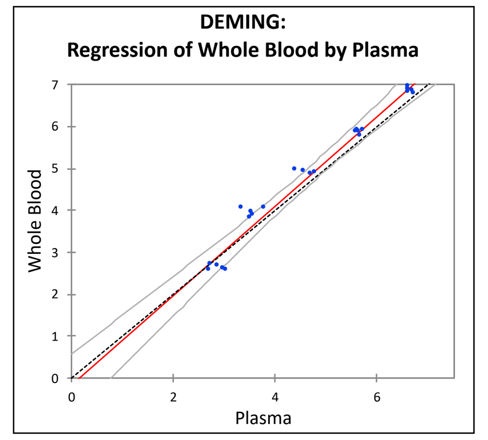
NeuMoDx EBV Quant Assay Interference - Endogenous Substances		
Endogenous	Average Conc. (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)
Albumin (120 mg/mL)	3.11	0.07
Bilirubin (0.02 mg/mL)	3.16	0.02
Hemoglobin (3 mg/mL)	3.12	0.07
Triglycerides (5 mg/mL)	3.21	0.03

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

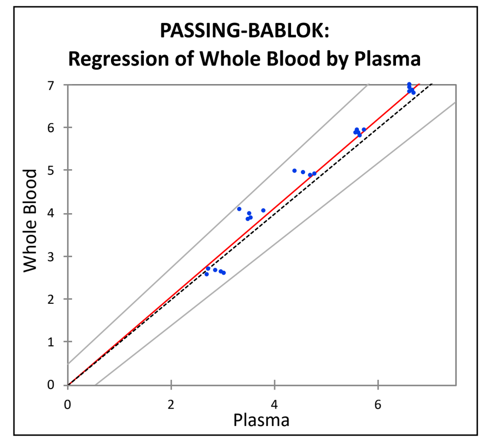
Equivalency Between Matrices

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 amounts 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.



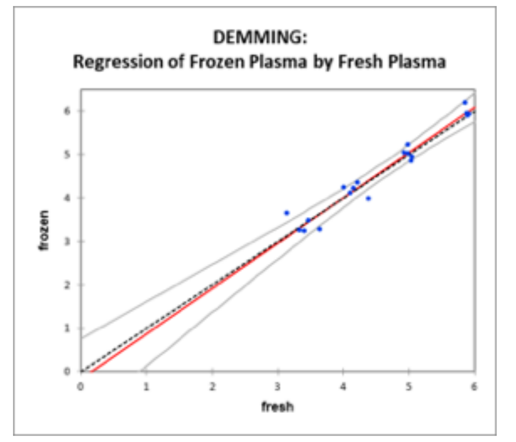
Deming Analysis
Intercept
-0.18 95% CI (-0.66, 0.30)
Slope coefficient
1.07 95% CI (0.99, 1.15)
Correlation coefficient
R2=0.99



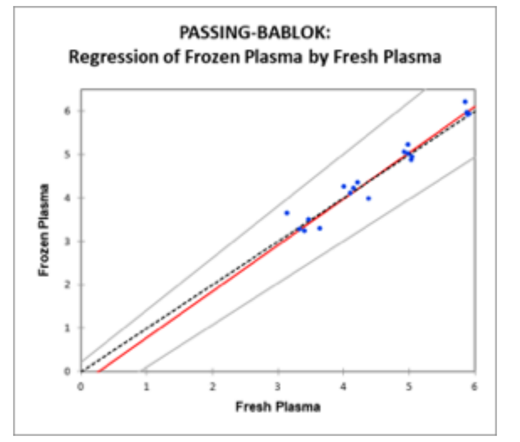
Passing-Bablok Analysis
Intercept
-0.004 95% CI (-0.51, 0.48)
Slope coefficient
1.04 95% CI (0.95, 1.13)
p-value
0.25

EBV PLASMA FRESH TO FROZEN EQUIVALENCY

The equivalency of the NeuMoDx EBV Test between fresh and frozen K2EDTA plasma was evaluated by spiking four levels of Exact EBV into EBV-negative plasma to span the linear range of the test. Specimens were then processed on the System. After processing, samples were frozen at -20° > C for > 24 hours. After freezing samples were thawed and reprocessed on the System. The NeuMoDx EBV Test demonstrated excellent quantitative correlation between fresh and frozen plasma specimens with Deming and Passing-Bablok linear regression analysis.



Deming Analysis
Intercept
-0.17 95% CI (-0.84, 0.49)
Slope coefficient
1.04 95% CI (0.92, 1.17)
Correlation coefficient
0.98



Passing-Bablok Analysis
Intercept
-0.29 95% CI (-0.87, 0.22)
Slope coefficient
1.06 95% CI (0.96, 1.20)
p-value
0.40

CONCLUSIONS

The NeuMoDx EBV Test demonstrated excellent performance and is well suited for implementing viral load monitoring using both plasma and whole blood specimens. *For Research Use Only. Not for use in diagnostic procedures.*

ACKNOWLEDGEMENTS

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