PERFORMANCE OF A QUANTITATIVE CYTOMEGALOVIRUS ASSAY ON FULLY AUTOMATED MOLECULAR DIAGNOSTIC SYSTEMS

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BACKGROUND

Human cytomegalovirus (CMV) is a betaherpesvirus commonly encountered during childhood. In immunocompromised individuals, especially transplant patients, CMV is one of the most significant causes of opportunistic infection and associated morbidity and mortality. Quantitative viral load measurements are among the most useful diagnostic tests available for measuring CMV DNA in such patients and are crucial to monitoring disease progression, efficacy of therapies, as well as detecting drug resistant mutants and identifying relapse upon discontinuation of antiviral therapy. The NeuMoDx CMV Assay is a "Sample to result" *in-vitro* diagnostic test offered on two NeuMoDx Molecular Systems – the NeuMoDx 288 and the NeuMoDx 96 – which automate and integrate DNA extraction, amplification, and results interpretation to provide rapid and accurate quantitative results.

METHODS

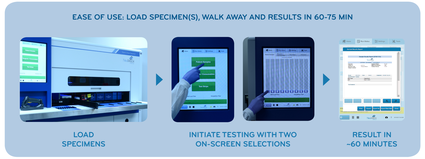
The objective of this study was to characterize performance of the NeuMoDx CMV Assay across key analytical metrics on both systems. These studies included characterization of linearity, sensitivity, precision, cross-reactivity, inclusivity, time to first result, and the effect of interfering substances. Additionally a method correlation study was performed to assess concordance with a reference test using remnant clinical specimens. Analytical sensitivity was determined using the 1st WHO International Standard for CMV and the lower and upper limits of quantitation (LLoQ/ULoQ) were determined using the TAE ≤1.0 criterion. Secondary standards were traced to the 1st WHO CMV International Standard and used for the additional testing.

RESULTS

The NeuMoDx CMV Assay demonstrated excellent linearity across clinically relevant >6 Log dynamic range with a slope of 0.998. Evaluation of analytical sensitivity resulted in a LoD and LLoQ of 20 IU/mL. Excellent quantitative precision was demonstrated across 3 systems over 12 days. The time to first result (turnaround time) for the NeuMoDx CMV Assay was ~60 min. No cross-reactivity was detected against any of the 35 non-target pathogens and no interference was observed against any of the non-target pathogens or the 33 endogenous and exogenous agents tested. A method correlation study conducted between the NeuMoDx CMV Assay and the reference test showed excellent linear correlation and a bias of 0.24 Log_{10} IU/mL.

NeuMoDx Molecular System Streamlined Testing

WORKFLOW



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.

CMV Analytical Sensitivity (LoD & LLoQ)

LIMIT OF DETECTION (LoD) AND LOWER LIMIT OF QUANTITATION (LLoQ)

The Limit of Detection of the NeuMoDx CMV Assay was determined with pooled CMV negative plasma spiked with 1st WHO International Standard (variant gB1) at six different concentrations including negative samples.

The limit of detection of CMV was determined to be 17.7 IU/mL based on Probit style analysis (rounded up to 20 IU/mL) and the calculated LLoQ was determined to also be 20 IU/mL.

NeuMoDx CMV Assay Limit of Detection					
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit)
50	1.70	108	108	100%	17.7 IU/mL 95% CI (13.2-21.0 IU/mL)
30	1.48	108	107	99%	
25	1.40	108	106	98%	
20	1.30	108	105	97%	
15	1.18	108	99	92%	
NEG	N/A	108	0	0%	

Limit of Detection of the NeuMoDx CMV Assay. Probit analysis from the data in the above table was used to determine the LoD of the CMV target to be 17.7 IU/mL with a 95% CI of (13.8 - 21). The LoD value was rounded up to 20 IU/mL.

NeuMoDx CMV Assay Lower Limit of Quantitation						
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	% Positive	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)	LLoQ (IU/mL)
50	1.70	100%	0.05	0.16	0.37	20 IU/mL
30	1.48	99.1%	0.14	0.24	0.62	
25	1.40	98%	0.17	0.19	0.55	
20	1.30	97%	0.27	0.22	0.72	
15	1.18	92%	0.35	0.21	0.78	

Lower limit of quantitation (LLoQ) of NeuMoDx CMV Assay. The lowest target level detected at a rate ≥95% AND with TAE (bias+2*SD) ≤ 1.0 was used to determine the LLoQ. Although the calculated TAE values were < 1.0 for all levels tested, the LLoQ of the NeuMoDx CMV Assay was determined to be 20 IU/mL where there was > 95% positivity, which corresponds to LoD.

GENOTYPE SENSITIVITY

The LoD and LLoQ of the NeuMoDx CMV Assay previously defined for gB1 was confirmed across genotypes using pooled CMV negative plasma spiked with different CMV genotypes (gB1-gB4) at 20 IU/mL (1.3 log_{10} IU/mL).

NeuMoDx CMV Assay LoD and LLoQ at 20 IU/mL (1.3 Log_{10} IU/mL)						
Genotype	N	# Positive	Detection Rate (%)	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)
gB1	110	106	96.4%	0.28	0.26	0.80
gB2	60	59	98.3%	0.22	0.23	0.67
gB3	60	59	98.3%	0.23	0.22	0.67
gB4	60	57	95.0%	0.30	0.30	0.90

CMV genotype inclusivity. The NeuMoDx CMV Assay accurately detected all relevant genotypes of CMV in plasma at 20 IU/mL. The overall LoD and LLoQ of the NeuMoDx CMV Assay is 20 IU/mL.

CMV Linearity

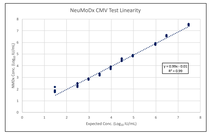
LINEAR RANGE AND UPPER LIMIT OF QUANTITATION (ULoQ)

The linearity of the NeuMoDx CMV Assay was determined by diluting either the NeuMoDx Encapsulated CMV Positive Control or a CMV Positive Sample (Exact Diagnostics LLC, Fort Worth, TX) (gB1 variants) in pooled CMV negative plasma to create a panel spanning >6 Log_{10} units of CMV concentration ranging from 8.0 Log_{10} IU/mL to 1.7 Log_{10} IU/mL.

Data from this study showed that the NeuMoDx CMV Assay demonstrated excellent linearity across >6 Log_{10} units, with a ULoQ determined at 8.0 Log_{10} IU/mL.

NeuMoDx CMV Assay Linearity							
Target Conc. (Log_{10} IU/mL)	N	% Detection	Mean Conc. (Log_{10} IU/mL)	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)	Calculated Linear Fit
8.0	36	100	8.05	0.05	0.09	0.23	7.88
7.0	36	100	6.99	0.01	0.07	0.16	6.90
6.7	36	100	6.74	0.04	0.09	0.21	6.60
6.0	36	100	5.96	0.04	0.09	0.22	5.91
5.7	36	100	5.71	0.01	0.10	0.22	5.61
4.7	36	100	4.65	0.05	0.11	0.28	4.63
3.7	36	100	3.61	0.09	0.15	0.38	3.64
2.7	36	100	2.68	0.02	0.12	0.27	2.65
1.7	36	100	1.80	0.10	0.19	0.48	1.67

Linear range of the NeuMoDx CMV Assay. The NeuMoDx CMV Assay is linear over more than 6 Log_{10} units.

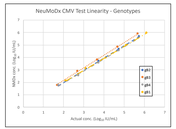


Linearity of the NeuMoDx CMV Assay. A second linearity panel was prepared using CMV reference materials and secondary standards (1st WHO International Standard, CMV NIST Reference Standard, Exact CMV Positive Sample, and the NeuMoDx Encapsulated CMV Positive Control) to span the linear range. The NeuMoDx CMV Assay shows excellent correlation over more than 6 Log_{10} units.

LINEARITY ACROSS CMV GENOTYPES

The linearity of the NeuMoDx CMV Assay was determined by diluting each CMV genotype in pooled CMV negative plasma to create a panel spanning the linear range of the NeuMoDx CMV Assay from ~6 Log_{10} IU/mL to ~2 Log_{10} IU/mL (depending on concentration of each genotype).

Data from this study showed that the NeuMoDx CMV Assay demonstrated excellent linearity across the >4 Log_{10} units.



CMV Genotype Linearity. Linearity confirmed across 4 CMV variants. (Source: Exact Diagnostics LLC, Fort Worth, TX)

Cross-Reactivity and Interference

The performance of the NeuMoDx CMV Assay was assessed in presence of phylogenetically similar organisms, commensal organisms, medications, disease state samples and high levels of endogenous samples for potential cross-reactivity and/or interference effect.

None of the phylogenetically similar organisms (non-target organisms) were detected by the NeuMoDx CMV Assay, showing excellent analytical specificity. Additionally, the NeuMoDx CMV Assay had minimal deviation of quantitation from CMV control samples, with no significant interference caused by the presence of any of the substances listed.

Non-Target Organisms	Exogenous Substances (Medications)
Adenovirus type 5	Azathioprine
BK Polyomavirus	Cyclosporine
Herpes Simplex Virus type-1	Foscarnet
Herpes Simplex Virus type-2	Ganciclovir
Epstein–Barr virus	Valganciclovir hydrochloride
Human Herpes Virus type-6	Prednisone
Human Herpes Virus type-7	Cidofovir
Human Herpes Virus type-8	Cefotetan
JC Virus	Cefotaxime
Hepatitis B Virus	Fluconazole
Hepatitis C Virus	Mycophenolate mofetil
HIV 1	Mycophenolate sodium
HIV 2	Piperacillin
Human Papillomavirus 16	Sirolimus/rapamycin
Human Papillomavirus 18	Tazaobactam
Parvovirus B19	Trimethoprim
Varicella-Zoster Virus	Vancomycin
Chlamydia trachomatis	Tacrolimus
Clostridium perfringens	Everolimus
Enterococcus faecalis	Clavulanate potassium
Escherichia coli	Famotidine
Klebsiella pneumoniae	Sulfamethoxazole
Neisseria gonorrhoeae	Valacylovir
Listeria monocytogenes	Letermovir
Mycobacterium avium	Ticarcillin disodium
Mycoplasma pneumoniae	Leflunomide
Propionibacterium acnes	**Disease State Samples**
Salmonella typhimurium	Systemic Lupus Erythematosus (SLE)
Staphylococcus aureus	Antinuclear Antibody (ANA)
Staphylococcus epidermidis	Rheumatoid Arthritis (RA)
Streptococcus pneumoniae	**Endogenous Substances**
Streptococcus pyogenes	Bilirubin
Aspergillus niger	Protein (albumin)
Candida albicans	Hemoglobin
Cryptococcus neoformans	Triglycerides

Precision

The Within Lab Precision of the NeuMoDx CMV Assay was determined by testing replicates of a 4 member panel of CMV on 2 NeuMoDx 288 Systems and 1 NeuMoDx 96 System over 12 days, with 2 runs per day.

The precision both Within-Run and Across-Runs was characterized and the standard deviation for both was determined to be ≤ 0.15 Log_{10} IU/mL.

NeuMoDx CMV Assay Within Lab Precision							
Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} U/mL)	N	Within System SD	Within Day SD	Within Run SD	(Overall) Within Lab SD
1	5.7	5.64	216	0.09	0.09	0.07	0.13
2	4.7	4.58	216	0.10	0.10	0.08	0.14
3	3.7	3.60	216	0.09	0.09	0.07	0.12
4	2.7	2.62	216	0.13	0.13	0.10	0.15

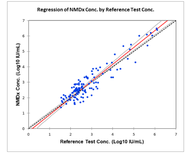
Method Correlation

The clinical sensitivity, specificity, and quantitative performance of the NeuMoDx CMV Assay was assessed against 3 CE/FDA approved comparator tests by processing 286 clinical specimens from CMV-tested patients.

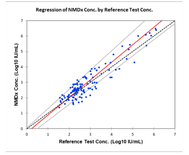
Testing was performed internally at NeuMoDx through a single-blinded study using clinical samples obtained from reference laboratories running Cobas Amplirep/Cobas CMV Taqman Assay, Cobas CMV Assay on Roche 6800 and CMV MGB Alert on Elitech InGenius.

A total of 123 clinical positive plasma specimens within the linear range from the study were used to generate the linear regression.

The NeuMoDx CMV Assay demonstrated excellent concordance of qualitative results and quantitative correlation with the reference tests.



Deming Analysis
Intercept
-0.18 95%CI (-0.39,0.03)
Slope coefficient
1.1 95%CI (1.0,1.2)



Passing-Bablock Analysis
Intercept
-0.24 95%CI (-0.51,0.06)
Slope coefficient
1.1 95%CI (1.0,1.2)

Clinical Quantitative Correlation of the NeuMoDx CMV Assay. The NeuMoDx CMV Assay demonstrated excellent quantitative correlation with an overall bias of either -0.18 Log_{10} IU/mL or -0.24 Log_{10} IU/mL for this quantitative study using either Deming linear regression analysis or Passing-Bablock analysis respectively.

	Reference Test Positive	Reference Test Negative	Total
NeuMoDx CMV Quant Assay Positive	129	4	133
NeuMoDx CMV Quant Assay Negative	6	144	150
Total	135	148	283
SENSITIVITY = 95.6% 95% CI (90.2%, 98.2%) SPECIFICITY = 97.3% 95% CI (92.8%, 99.1%)			

Clinical Qualitative Correlation of the NeuMoDx CMV Assay. The NeuMoDx CMV Assay demonstrated excellent concordance with the reference tests as shown by Sensitivity of 95.6% with a 95% CI of 90.2% to 98.2% and Specificity of 97.3% with a 95% CI of 92.8% to 99%.

CONCLUSIONS

The NeuMoDx CMV Assay demonstrated excellent performance across all analytical metrics, while demonstrating excellent correlation to a current reference test. Additionally, the NeuMoDx Molecular Systems provided an excellent workflow for implementing viral load monitoring for immunocompromised patients in the critical organ transplant patient population.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help and support provided by all members of the NeuMoDx team. We would also like to thank our collaborators for their invaluable assistance in providing access to clinical specimens for testing and evaluation.

The NeuMoDx CMV Assay is not for sale in the United States.