QUANTITATIVE DETECTION OF CYTOMEGALOVIRUS ON NEUMODX MOLECULAR SYSTEMS

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BACKGROUND

Cytomegalovirus (CMV) is one of the major causes of opportunistic infection, and associated morbidity and mortality, in immunocompromised patients such as organ transplant recipients. Quantitative PCR methods are among the most useful diagnostic tests available for measuring CMV replication in such patients. Such viral load measurements are crucial to monitoring disease progression, efficacy of antiviral therapies, as well detecting drug resistant mutants and identifying relapse upon discontinuation of antiviral therapy. The NeuMoDx CMV Test is a "sample to result" in-vitro diagnostic assay offered on two NeuMoDx Molecular Systems – the NeuMoDx 288 and the NeuMoDx 96 - which automate and integrate the extraction, amplification, and results interpretation using quantitative PCR. Performance of the NeuMoDx CMV Test on both systems was characterized and compared to an existing reference test.

METHODS

The objective of this study was to characterize performance of the NeuMoDx CMV Test across key performance metrics. Analytical studies – sensitivity, cross-reactivity, inclusivity, interfering substances, and a pilot method comparison study – were performed. Analytical sensitivity was determined via a Probit-style analysis using the 1st WHO International CMV Standard, and the lower limit of quantitation (LLoQ) was determined using the Total Analytic Error <1.0 criterion. Specificity was tested by screening >30 phylogenetically similar or cohabitating organisms while typical interfering moieties were tested to screen for interfering effects. Finally, a small-scale method correlation study involving 100 specimens was performed to assess concordance with a reference test.

RESULTS

Evaluation of analytical sensitivity shows the NeuMoDx CMV Test was able to detect <40 IU/mL with at least 95% sensitivity, and LLoQ was also determined to be <40 IU/mL. The NeuMoDx CMV Test demonstrated excellent linearity across clinically relevant dynamic range (R2 > 0.99 across 6 Logs), as well as precision across systems, days, and reagent lots. Turnaround time (TAT) for the NeuMoDx CMV Test was ~65 min and the 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. The comparison study conducted between the NeuMoDx CMV Test and the reference test showed excellent linear correlation and a bias of < 0.5 Log_{10} IU/mL.

CMV Analytical Sensitivity

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

The Limit of Detection of the NeuMoDx CMV Test was determined with pooled CMV negative plasma spiked with 1st WHO International Standard (variant gB1) at six different levels 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 be the same at 20 IU/mL.

NeuMoDx CMV Test Limit of Detection

Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
50	1.70	108	108	100%	17.7 IU/mL 95% CI (13.8-21.0 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 Test. 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 Test LLoQ

Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)	LLoQ (IU/mL)
50	1.70	108	0.05	0.16	0.37	20 IU/mL
30	1.48	108	0.14	0.24	0.62	
25	1.40	108	0.17	0.19	0.55	
20	1.30	108	0.27	0.22	0.72	
15	1.18	108	0.35	0.21	0.78	

Lower limit of quantitation (LLoQ) of NeuMoDx CMV Test. 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 Test was determined to be 20 IU/mL, which corresponds to LoD.

GENOTYPE SENSITIVITY

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

NeuMoDx CMV Test 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	0.28	0.26	0.80
gB2	60	59	98	0.22	0.23	0.67
gB3	60	59	98	0.23	0.22	0.67
gB4	60	57	95	0.30	0.30	0.90

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

CMV Linearity

LINEAR RANGE AND UPPER LIMIT OF QUANTITATION (ULoQ)

The linearity of the NeuMoDx CMV Test 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 1.5 Log_{10} IU/mL.

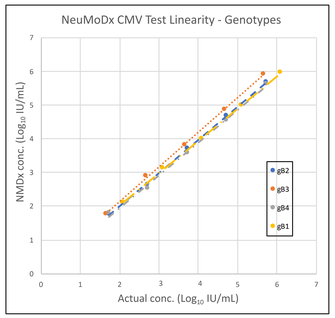
Data from this study showed that the NeuMoDx CMV Test demonstrated excellent linearity across >6 Log_{10} units, with a ULoQ determined at 8.0 Log_{10} IU/mL.

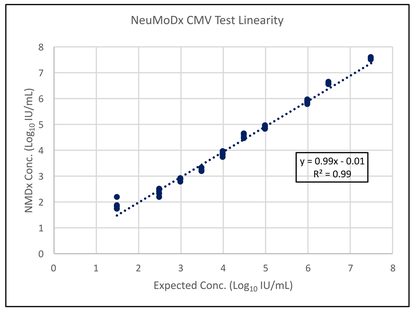
NeuMoDx CMV Test Linearity

Target Conc. (Log_{10} IU/mL)	N	# Positive	% Detection	Mean Conc. (Log_{10} IU/mL)	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)	Calculated Linear Fit
8	36	36	100	8.05	0.05	0.09	0.23	7.88
7	36	36	100	6.99	0.01	0.07	0.16	6.90
6.7	36	36	100	6.74	0.04	0.09	0.21	6.60
6	36	36	100	5.96	0.04	0.09	0.22	5.91
5.7	36	36	100	5.71	0.01	0.10	0.22	5.61
4.7	36	36	100	4.65	0.05	0.11	0.28	4.63
3.7	36	36	100	3.61	0.09	0.15	0.38	3.64
2.7	36	36	100	2.68	0.02	0.12	0.27	2.65
1.7	36	36	100	1.80	0.10	0.19	0.48	1.67

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

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





LINEARITY ACROSS CMV GENOTYPES

The linearity of the NeuMoDx CMV Test was determined by diluting each CMV genotype in pooled CMV negative plasma to create a panel spanning the linear range of the NeuMoDx CMV Test 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 Test 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)

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.

Cross-Reactivity and Interference

The performance of the NeuMoDx CMV Test was assessed in presence of phylogenetically similar organisms, co-habitating organisms and drugs for potential cross-reactivity and/or interference effect.

Non-Target Organisms	Exogenous Substances
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	**Endogenous Substances**
Salmonella typhimurium	Systemic Lupus Erythematosus (SLE)
Staphylococcus aureus	
Staphylococcus epidermidis	Antinuclear Antibody (ANA)
Streptococcus pneumoniae	Rheumatoid Arthritis (RA)
Streptococcus pyogenes	Bilirubin
Aspergillus niger	Protein (albumin)
Candida albicans	Hemoglobin
Cryptococcus neoformans	Triglycerides

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

Precision

The Within Lab Precision of the NeuMoDx CMV test was determined by testing a 4 member panel of CMV on multiple NeuMoDx Molecular Systems across multiple runs performed across multiple days.
The precision 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 Test Within Lab Precision

Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	N	Within System SD	Within Day SD	Within Run SD	(Overall) Within Lab SD
1	5.7	5.64	180	0.09	0.08	0.06	0.13
2	4.7	4.57	180	0.10	0.10	0.08	0.14
3	3.7	3.60	180	0.09	0.09	0.07	0.12
4	2.7	2.62	180	0.12	0.11	0.10	0.15

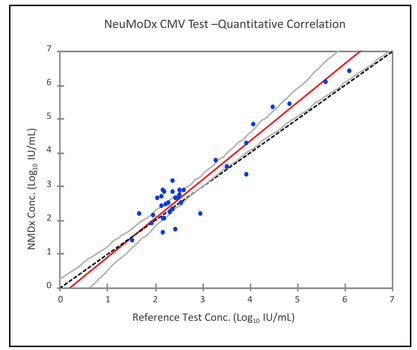
Method Correlation

The clinical sensitivity and specificity and quantitative performance of the NeuMoDx CMV Test was assessed against 3 CE/FDA approved comparator tests by testing 95 clinical specimens from CMV-tested patients.

A total of 35 clinical positive plasma specimens within the linear range were used to generate the linear regression.

Testing was performed internally at NeuMoDx through a single-blinded study using clinical samples obtained from reference laboratories.

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



Deming Analysis	
Intercept	-0.27 95%CI (-0.69,0.14)
Slope Coefficient	1.15 95%CI (1.02,1.29)

Clinical Quantitative Correlation of the NeuMoDx CMV Test. The NeuMoDx CMV Test demonstrated excellent quantitative correlation with an overall bias of -0.27 Log_{10} IU/mL for this preliminary quantitative study using the Deming linear regression analysis tool.

CONCLUSIONS

The NeuMoDx CMV Test provides an excellent workflow for implementing viral load monitoring for immunocompromised patients. Performance across analytical metrics was superior to existing tests while demonstrating excellent correlation to a current reference test.

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