RAPID AND FULLY AUTOMATED IMPLEMENTATION OF LABORATORY DEVELPED TESTS ON THE NEUMODX MOLECULAR SYSTEM

M Mastronardi*, L Gong, C Couture, A McCarthy, J Zhu, M Carey, B Wu, S Brahmasandra, NeuMoDx Molecular, Ann Arbor, MI

BACKGROUND

Most fully integrated platforms for molecular diagnostics do not allow automation of end-user developed assays, leading to significant inefficiencies in implementation of Laboratory Developed Tests (LDTs), such as manual batch processing spanning multiple pieces of equipment and requiring an isolated high-complexity lab. The NeuMoDx Molecular System helps overcome these significant drawbacks by integrating the discrete processes of nucleic acid extraction, purification, reaction preparation, amplification, detection, and results interpretation within a system architecture that enables extremely easy and efficient automation of LDTs. The objective of this study was to demonstrate the LDT implementation capabilities of the NeuMoDx Molecular System using a model qualitative RNA assay from nasopharyngeal swab specimens and a model quantitative DNA assay from plasma specimens.

MATERIALS/METHODS

A *Cytomegalovirus* real-time PCR assay and a *Respiratory syncytial virus* real-time RT-PCR assay was used to assess the efficacy of automated LDT implementation. General purpose reagents and consumables, including universal Extraction Plates and Lysis Buffers, as well as PCR and RT-PCR Master Mix Test Strips containing the necessary reagents to amplify, but only detect a sample process control were loaded onto the System worktable. Assay-specific primers and probes were provided in separate empty test strips and also loaded on to the system worktable prior to test initiation. Various analytical performance parameters for both tests were determined to assess performance.

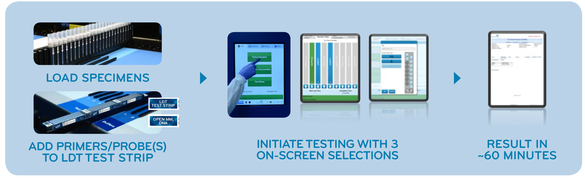
RESULTS

Turnaround time for the entire test was ~60 minutes for CMV and ~80 minutes for RSV. The Limit of Detection (LoD) of the RSV LDT was determined to be ~1 $TCID_{50}$/mL. The LoD and Lower Limit of Quantitation (LLoQ) of the CMV LDT were both determined to be <40 IU/ mL. The CMV LDT demonstrated excellent linearity across clinically relevant measurement dynamic range (R2 0.99; 6-7 logs). Excellent reproducibility was demonstrated across multiple days and systems for both RSV and CMV LDTs. All the reagents used in the study were ambient temperature stable and demonstrated in-use shelf life of >14 days.

NeuMoDx Molecular System Streamlined Testing for LDT Tests

NeuMoDx 288
Molecular System

LDT WORKFLOW



◄ **Large walkaway window of up to 288 samples with room temperature stable onboard reagents.**

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

FEATURES

- **Integrated Operation:** Integrates all steps of molecular diagnostics starting from raw clinical specimens to providing real-time PCR results in a fully automated process
- **True Random Access:** Unlimited ability to mix specimen types and tests
- **High Throughput:** ~400 DNA tests in an 8 hour shift
- **Fast Time to First Results:** <60 min for DNA Targets
- **Large Walk-Away Window:** Up to 288 samples
- **Continuous Loading:** Specimens and Reagents can be loaded/unloaded at any time
- **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

CMV as a Model for Quantitative LDT Implementation

Traceability to WHO Standard

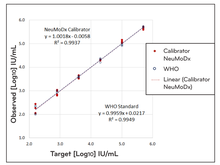
Traceability of the NeuMoDx CMV Calibrators to the 1st WHO International CMV Standard

The traceability of NeuMoDx CMV calibrators to the 1st WHO International Standard was characterized by testing the NeuMoDx CMV calibrators and WHO standard at similar levels (from 2.2 to 5.7 Log10 IU/mL) using LDT implementation on the NeuMoDx 288 Molecular System. The two materials behaved similarly and demonstrated a co-linear performance across the range tested.

Analytical Sensitivity

Limit of Detection and Lower Limit of Quantitation

The Limit of Detection (LoD) of the NeuMoDx CMV LDT was determined by evaluating 3 low levels of NeuMoDx CMV Calibrators spiked into CMV-negative EDTA plasma. The testing was performed with 30 replicates at each level across two NeuMoDx 288 Molecular Systems. The LoD was determined to be ~ 14 IU/mL based on Probit style analysis) for the NeuMoDx CMV LDT. The Lower Limit of Quantitation (LLoQ), defined as the lowest level tested yielding a Total Analytical Error (TAE) of ≤1, was 37.7 IU/mL.



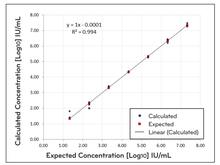
Target Conc. IU/mL	n	# Positive	% Positive	LoD (Probit)	LLoQ TAE ≤1
50	29	29	100%	14 IU/mL	37.7 IU/mL
1	29	29	100%		
0.5	30	23	76.7%		

Table 2. Limit of Detection and Lower Limit of Quantitation of the NeuMoDx CMV LDT. Probit analysis of the data in the above table was used to determine the LoD of the CMV Target to be 14 IU/mL. The lowest level detected with TAE ≤1.0 was determined to be 37.7 IU/mL.

Linearity

CMV Linearity

The linearity of NeuMoDx CMV LDT was evaluated with a dilution series of NeuMoDx CMV calibrator (Merlin strain) in pooled CMV-negative plasma to create a 7 member panel spanning the intended linear range. The NeuMoDx CMV LDT test demonstrated excellent linearity across all levels tested (2.15E1 IU/mL to 2.15E7 IU/mL in EDTA plasma) and was capable of quantitating CMV across this linear range with a maximum deviation of ±0.15 Log10 IU/mL. The maximum deviation from a slightly better fitting non-linear regression fit was ±0.14 Log10 IU/mL.



Target Conc. IU/mL	Target Conc. (Log_{10} IU/mL)	Mean Titer (Log_{10} IU/mL)	SD Log 10 Titer (IU/mL)	Bias	Predicted Linear Fit	Deviation
2.15E+01	1.33	1.61	0.27	0.28	1.33	0.28
2.15E+02	2.33	2.16	0.18	-0.17	2.33	-0.17
2.15E+03	3.33	3.34	0.07	0.01	3.33	0.01
2.15E+04	4.33	4.34	0.04	0.01	4.33	0.01
2.15E+05	5.33	5.31	0.05	-0.02	5.33	-0.02
2.15E+06	6.33	6.37	0.11	0.03	6.33	0.03
2.15E+07	7.33	7.34	0.11	0.01	7.33	0.01

Table 1. Linearity of the NeuMoDx CMV LDT. Maximum deviation from a slightly better fitting nonlinear fit was 0.17 Log10 IU/mL

Reproducibility

CMV Reproducibility

Reproducibility of the NeuMoDx CMV LDT was demonstrated across 3 days by testing a 3 member panel of CMV on two NeuMoDx Molecular Systems. The panel was prepared using the CMV Calibrator, and covered a range from 5.46E2 to 5.46E6 IU/mL. The reproducibility within-run and across runs was characterized and the Standard Deviations (SD) are shown in Table 3.

Panel Member	Target Conc. (Log10 IU/mL)	Mean Conc. (Log10 IU/mL)	n	Within Run SD	Across Runs SD	Overall SD
1	6.74	6.65	36	0.09	0.24	0.25
2	4.74	4.57	36	0.08	0.26	0.27
3	2.74	2.62	36	.010	0.29	0.31

Table 3. The NeuMoDx CMV LDT demonstrated excellent reproducibility across target levels and Systems.

RSV as a Model for Qualitative LDT Implementation

Reproducibility

RSV Reproducibility

Reproducibility of the NeuMoDx RSV LDT was demonstrated across 3 days by testing a 3 member panel of RSV on two NeuMoDx Molecular Systems. The panel was prepared using viral lysate from ATCC (Long strain), and covered a range from 2E1 to 2E6 IU/mL. The reproducibility within-run and across runs was characterized and the Standard Deviations (SD) are shown in Table 4.

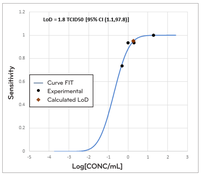
Panel Member	Target Level ($TCID_{50}$)	Mean Ct	n	Within Run SD	Across Runs SD	Overall SD
1	2E+01	31.2	36	1.03	0.47	1.01
2	2E+03	24.0	36	0.75	0.59	0.74
3	2E+05	17.5	36	0.56	0.71	0.57

Table 4. The NeumoDx RSV LDT demonstrated excellent reproducibility across target levels and systems.

Analytical Sensitivity

RSV Limit of Detection

The Limit of Detection (LoD) of the NeuMoDx RSV LDT was determined by evaluating 4 low levels of RSV (Long strain) spiked into UVT media containing nasal swabs. The testing was performed with 30 replicates at each level across two NeuMoDx 288 Molecular Systems. The LoD was determined to be 1.85 TCID50/mL based on Probit style analysis) for the NeuMoDx RSV LDT.



Target Conc. $TCID_{50}$/mL	n	# Positive	% Positive	LoD (Probit)
20	30	30	100%	1.85 $TCID_{50}$/mL
2	30	28	97%	
1	30	28	97%	
0.5	30	22	73%	

Table 5. Limit of Detection and Lower Limit of Quantitation of the NeuMoDx RSV LDT. Probit analysis of the data in the above table was used to determine the LoD of the RSV Target to be 1.85 $TCID_{50}$/mL.

CONCLUSIONS

The study demonstrated the extreme ease of LDT implementation as well as excellent performance for both qualitative (RSV) and quantitative (CMV) tests as implemented on the NeuMoDx Molecular System.

ACKNOWLEDGMENTS

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