QUANTITATIVE DETECTION OF HCV USING A FULLY INTEGRATED "SAMPLE TO RESULT" MOLECULAR DIAGNOSTIC SYSTEM

TUESDAY-19

Elizabeth Craig*, Jessica Zhu, Maureen Carey, Catherine Couture, Hui Lin Lee, Michelle Mastronardi, Betty Wu, Sundu Brahmasandra, NeuMoDx Molecular, Ann Arbor, MI

BACKGROUND

Determining Hepatitis C Virus (HCV) RNA levels in plasma and/or serum is an important tool to characterize viral loads in infected patients to monitor disease progression, efficacy of antiviral therapies, as well as to detect drug resistant mutants and identify relapse upon discontinuation of an antiviral therapy. The NeuMoDx HCV Test is an *in-vitro* diagnostic assay incorporating a universal nucleic acid isolation chemistry enabling extraction of qPCR ready RNA from serum and plasma specimens, combined with a sensitive quantitative rt-PCR assay to deliver highly accurate results in a completely automated, "random access" manner on the NeuMoDx Molecular System. In addition, all reagents and disposables are room temperature stable and are intended to remain on-board the system to provide a seamless, on-demand testing workflow.

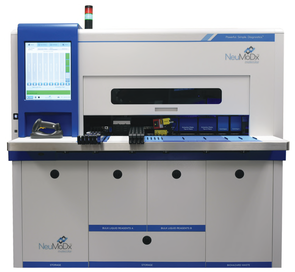
METHODS

The NeuMoDx Molecular System automates and integrates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using quantitative RT-PCR. The objective of this study was to test and report performance of the NeuMoDx HCV Test in key analytical performance metrics. Internal pre-analytical studies were performed to characterize the analytical sensitivity, linearity, precision, inclusivity, turnaround time, and results processing accuracy and are reported here.

RESULTS

The NeuMoDx HCV Test showed a detection limit and lower limit of quantification of 10 IU/mL and demonstrated excellent linearity across a 8 Log dynamic range (R^2>0.98). Reproducibility and precision across multiple systems, operators, and reagent lots was also demonstrated. The NeuMoDx HCV Test showed equivalent detection performance across all relevant HCV genotypes with a Turnaround time (TAT) of ~75 min. The results processing module incorporated for automated processing of data provides accurate quantitative results, and excellent concordance was demonstrated in a method correlation study conducted between the NeuMoDx HCV Test and a reference test. An R^2 >0.95 and bias of HCV viral load output from the two tests of less than 0.5 Log IU/mL was obtained.

NeuMoDx Molecular System Streamlined Testing



NeuMoDx 288 Molecular System

WORKFLOW

Ease of use: Load specimen(s), walk away and results in 75 min

LOAD SPECIMENS

INITIATE TESTING WITH TWO ON-SCREEN SELECTIONS

RESULT IN ~75 MINUTES

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/~300 RNA tests in an 8 hour shift
- **Fast Time to First Results:** <75 min for RNA 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

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.

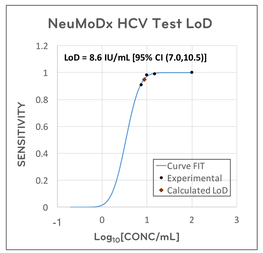
HCV Analytical Sensitivity (LoD & LLoQ)

The Limit of Detection of the NeuMoDx HCV Test was determined with pooled HCV negative plasma spiked with Acrometrix® HCV Control at three different levels including negative samples.

The Limit of Detection of HCV was determined to be 8.6 IU/mL based on Probit style analysis and the calculated LLoQ was determined to be 10 IU/mL.

The NeuMoDx HCV Test further demonstrated sensitivity by accurately detecting all eight HCV genotypes near the limit of detection.

NeuMoDx HCV Test Limit of Detection					
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
100	2	48	48	100%	8.6 (7.0,10.5)
15	1.18	118	117	99.1%	
10	1.00	123	121	98.3%	
7.5	0.88	124	113	91.1%	
0	-	48	0	0%	



Limit of Detection of the NeuMoDx HCV Test.
Probit analysis from the data in the above table was used to determine the LoD of the HCV target to be 8.6 IU/mL with a 95% CI of (7.0,10.5).

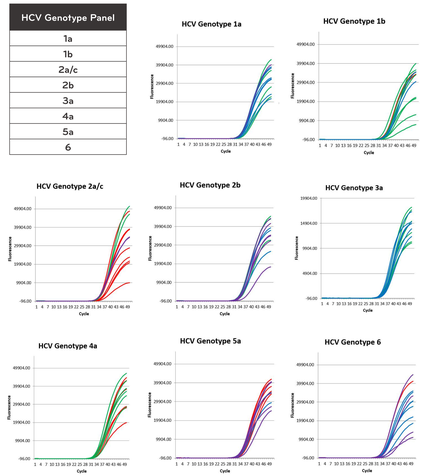
NeuMoDx HCV Test LLoQ						
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	Abs. Bias	Standard Deviation (SD)	Total Analytical Error (TAE)	LLoQ (IU/mL)
15	1.18	164	0.23	0.32	0.87	10
10	1	171	0.23	0.36	0.95	
7.5	0.88	169	0.34	0.44	1.22	

Lower limit of quantitation (LLoQ) of NeuMoDx HCV Test.
The lowest target level detected at a rate > 95% AND with TAE (bias+2*SD) ≤ 1.0 was used to determine the LLoQ. The LLoQ of the NeuMoDx HCV Test was determined to be ~10 IU/mL.

Genotype Coverage

HCV genotype inclusivity.
The NeuMoDx HCV Test accurately detected all eight clinically relevant genotypes of HCV (SeraCare HCV RNA Genotype AccuTrak™ Qualification Panel) with 100% amplification near LoD.

HCV Genotype Panel
1a
1b
2a/c
2b
3a
4a
5a
6

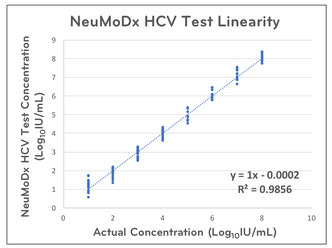


HCV Linearity

The linearity of the NeuMoDx HCV Test was determined by diluting either Acrometrix HCV RNA or Asuragen HCV Armored RNA® in pooled HCV negative plasma to create a panel spanning eight logs of HCV concentration ranging from 8 Log_{10} IU/mL to 1 Log_{10} IU/mL.

Data from this study showed that the NeuMoDx HCV Test demonstrated excellent linearity across the 8 logs with a maximum deviation from a slightly better fitting non-linear regression of 0.16 Log_{10} IU/mL.

NeuMoDx HCV Test Linearity						
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	Bias	Calculated Linear Fit	Calculated Best Non-Linear Fit	Deviation from Non-Linear Fit
1E+08	8.00	8.05	0.05	8	8.05	0.05
1E+07	7.00	7.10	0.10	7	7.08	0.08
1E+06	6.00	6.03	0.03	6	6.04	0.04
1E+05	5.00	4.87	0.13	5	5.00	0.00
1E+04	4.00	3.96	0.04	4	3.92	-0.08
1E+03	3.00	2.97	0.03	3	2.91	-0.09
1E+02	2.00	1.88	0.12	2	1.97	-0.02
1E+01	1.00	1.2	0.20	0.99	1.16	0.16



Linear range of the NeuMoDx HCV Test.
The NeuMoDx HCV Test is linear over 8 Log_{10} units with a maximum deviation of 0.16 Log_{10} IU/mL from a slightly better fitting non-linear regression.

Precision

The Within Lab Precision of the NeuMoDx HCV test was determined by testing a 5 member panel of HCV on multiple NeuMoDx Molecular Systems across multiple days.

The same concentrations of HCV were used across these consumables 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 ≤ 0.5 Log_{10} IU/mL.

NeuMoDx HCV Test Within Lab Precision						
Panel Member	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	N	Within Run SD	Across Runs SD	Overall SD
1	5.30	4.95	48	0.23	0.17	0.29
2	4.30	4.03	48	0.23	0.17	0.29
3	3.30	2.99	48	0.11	0.21	0.24
4	2.30	2.05	48	0.12	0.09	0.15
5	1.30	1.05	48	0.50	0.46	0.68

Precision of the NeuMoDx HCV Test.
The NeuMoDx HCV Test demonstrated excellent within laboratory precision calculated from the quantitative data across target levels, reagent lots, and Systems with a maximum overall standard deviation ≤ 0.68 Log_{10} IU/mL.

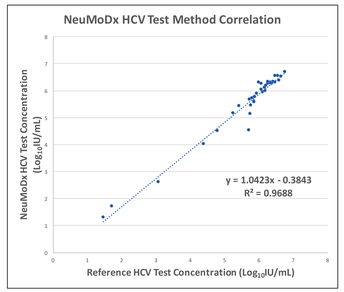
Method Correlation Study

The quantitative performance of the NeuMoDx HCV Test was assessed against an FDA approved comparator assay by testing clinical specimens from HCV infected patients.

A total of 36 clinical 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 a reference laboratory.

The NeuMoDx HCV test demonstrated excellent quantitative correlation with the reference test.



NeuMoDx HCV Test method correlation.
The NeuMoDx HCV Test demonstrates excellent correlation with the comparator HCV test results from a reference lab.

CONCLUSION

The NeuMoDx HCV Test is an extremely easy to use, rapid, automated molecular test for the sensitive and accurate viral load monitoring required for effective patient management of HCV infections.

ACKNOWLEDGMENTS

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.