QUANTITATIVE DETECTION OF HCV USING THE NEUMODX MOLECULAR DIAGNOSTIC SYSTEM

Jessica Zhu*, Elizabeth Craig, Maureen Carey, Catherine Couture, Michelle Mastronardi, Betty Wu, 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, as well as characterizing quantitative correlation to a reference test using split samples. The results of these studies are presented here.

RESULTS

The NeuMoDx HCV Test showed a detection limit of 7.5 IU/mL (95% CI of (6.4, 9.2)) and lower limit of quantification of 7.7 IU/mL using the 5th International WHO HCV Standard, with an overall LLoQ of 0.9 Log10 IU/mL for plasma and serum. A master calibration curve traceable to the 5th WHO HCV Standard as well as external calibrators (based on secondary standards traceable to the 5th WHO HCV Standard) were developed to provide accurate quantitative results across multiple systems and reagent lots. The NeuMoDx HCV Test demonstrated excellent linearity across an 8-10 Log dynamic range (R^2>0.99). The NeuMoDx HCV Test showed equivalent detection performance across all relevant HCV genotypes and a time to first results of ~80 min. No cross-reactivity or interference was observed against any of the pathogens or agents tested. The method correlation study performed with the NeuMoDx HCV test and using split samples demonstrated excellent concordance with 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.

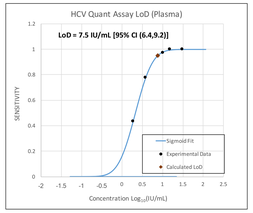
HCV Analytical Sensitivity (LoD & LLoQ)

PLASMA

The Limit of Detection of the NeuMoDx HCV Test was determined with pooled HCV negative plasma spiked with 5th WHO International Standard at seven different levels including negative samples.

The limit of detection of HCV was determined to be 7.5 IU/mL based on Probit style analysis and the calculated LLoQ was determined to be 7.7 IU/mL.

NeuMoDx HCV Test Limit of Detection					
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
30	1.48	108	108	100%	7.5 IU/mL 95% CI (6.4-9.2 IU/mL)
15	1.18	108	108	100%	
10	1.00	108	105	97%	
7.5	0.88	108	102	94%	
3.75	0.57	108	84	78%	
1.875	0.27	108	47	44%	
NEG	N/A	108	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 7.5IU/mL with a 95% CI of (6.4,9.2).

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)
30.00	1.48	108	0.07	0.32	0.71	7.7 IU/mL or 0.89 Log_{10} IU/mL
15.00	1.18	108	0.06	0.36	0.79	
10.00	1.00	108	0.07	0.35	0.77	
7.50	0.88	108	0.13	0.44	1.02	
3.75	0.57	108	0.51	0.43	1.38	
1.88	0.27	108	0.83	0.36	1.55	

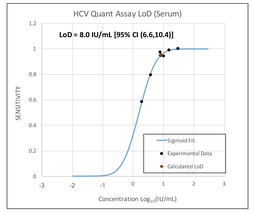
Lower limit of quantitation (LLoQ) of NeuMoDx HCV Test. The lowest target level detected at a rate > 95% AND with TAE (bias+2*SD) ≤ 1.0 was used to determine the LLoQ. The LLoQ of the NeuMoDx HCV Test was determined to be 7.7 IU/mL.

SERUM

The Limit of Detection of the NeuMoDx HCV Test was determined with pooled HCV negative plasma spiked with AcroMetrix High Control HCV (traceable to the WHO 5th IS) at seven different levels including negative samples.

The limit of detection of HCV was determined to be 8.0 IU/mL based on Probit style analysis and the calculated LLoQ was determined to be 8.4 IU/mL.

NeuMoDx HCV Test Limit of Detection					
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
30	1.48	108	108	100%	8.0 IU/mL 95% CI (6.6-10.4 IU/mL)
15	1.18	108	107	99%	
10	1.00	108	102	94%	
7.5	0.88	108	105	97%	
3.75	0.57	108	86	80%	
1.875	0.27	108	63	58%	
NEG	N/A	107	1	0.93%	



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 8.0IU/mL with a 95% CI of (6.6,10.4).

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)
30.00	1.48	108	0.08	0.30	0.69	8.4 IU/mL or 0.9 Log_{10} IU/mL
15.00	1.18	108	0.06	0.32	0.70	
10.00	1.00	108	0.14	0.36	0.85	
7.50	0.88	108	0.25	0.25	1.09	
3.75	0.57	108	0.59	0.58	1.76	
1.88	0.27	108	0.84	0.69	2.22	

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 8.4 IU/mL.

GENOTYPE SENSITIVITY

The LoD and LLoQ of the NeuMoDx HCV Test was confirmed with pooled HCV negative plasma spiked with different HCV genotypes at four different levels including negative samples.

HCV Genotype Panel	LoD (IU/mL)	LLoQ (IU/mL)
1	6.6	6.7
2	4.5	5.2
3	7.5	7.5
4	6.0	6.0
5	4.8	5.5
6	4.5	6.7

HCV genotype inclusivity. The NeuMoDx HCV Test accurately detected all six relevant genotypes of HCV in negative plasma with LoD at or under the WHO 5th IS LoD.

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 RNA tests in an 8 hour shift for the N288, ~100 RNA tests in an 8 hour shift for the N96
- **Fast Time to First Results:** ~75 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.

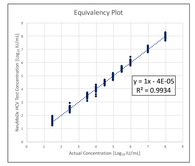
HCV Linearity

PLASMA

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 10 logs of HCV concentration ranging from 8.0 Log10 IU/mL to 1.5 Log10 IU/mL.

Data from this study showed that the NeuMoDx HCV Test demonstrated excellent linearity across the 10 logs.

NeuMoDx HCV Test Linearity				
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	Bias	Calculated Linear Fit
1.00E+08	8.00	8.00	0.00	8.00
1.00E+07	7.00	7.03	0.03	7.00
1.00E+06	6.00	6.04	0.04	6.00
3.16E+05	5.50	5.54	0.04	5.50
1.00E+05	5.00	4.95	0.05	5.00
3.15E+04	4.50	4.46	0.04	4.50
1.00E+04	4.00	3.97	0.03	4.00
3.16E+03	3.50	3.46	0.04	3.50
3.16E+02	2.50	2.52	0.02	2.50
3.16E+01	1.50	1.55	0.05	1.50



▲ **Linear range of the NeuMoDx HCV Test.** The NeuMoDx HCV Test is linear over 10 Log10 units.

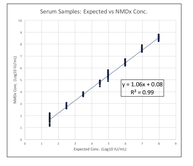
◀ **Linear range of the NeuMoDx HCV Test.** The NeuMoDx HCV Test shows excellent correlation over 10 Log10 units.

SERUM

The linearity of the NeuMoDx HCV Test was determined by diluting either Acrometrix HCV RNA or Asuragen HCV Armored RNA® in pooled HCV negative serum to create a panel spanning 8 logs of HCV concentration ranging from 8.0 Log10 IU/mL to 1.5 Log10 IU/mL.

Data from this study showed that the NeuMoDx HCV Test demonstrated excellent linearity across the 8 logs.

NeuMoDx HCV Test Linearity				
Target Conc. (IU/mL)	Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	Bias	Calculated Linear Fit
1.00E+08	8	8.54	0.54	8.48
1.00E+07	7	7.54	0.54	7.43
1.00E+06	6	6.46	0.46	6.38
1.00E+05	5	5.41	0.41	5.33
3.16E+04	4.5	4.77	0.27	4.80
3.16E+03	3.5	3.73	0.22	3.75
3.16E+02	2.5	2.74	0.24	2.70
3.16E+01	1.5	1.71	0.21	1.65



▲ **Linear range of the NeuMoDx HCV Test.** The NeuMoDx HCV Test is linear over 10 Log10 units.

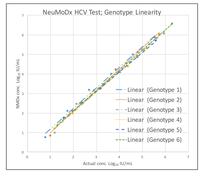
◀ **Linear range of the NeuMoDx HCV Test.** The NeuMoDx HCV Test shows excellent correlation over 8 Log10 units.

GENOTYPE SENSITIVITY

The linearity of the NeuMoDx HCV Test was determined by diluting each HCV genotype in pooled HCV negative plasma to create a panel spanning 6 logs of HCV concentration ranging from 6.5 Log10 IU/mL to 0.8 Log10 IU/mL (depending on concentration of each genotype).

Data from this study showed that the NeuMoDx HCV Test demonstrated excellent linearity across the 6 logs.

HCV Genotype Panel	LoD (IU/mL)	LLoQ (IU/mL)
1	y = 1.054x + 0.1325	0.9790
2	y = 1.0792x - 0.0748	0.9854
3	y = 1.0423x - 0.0439	0.9814
4	y = 1.0158x + 0.0292	0.9729
5	y = 0.9873x + 0.1524	0.9939
6	y = 1.0393x + 0.0396	0.9973



Linear range of the NeuMoDx HCV Test. Confirmed with 6 genotypes of HCV.

Cross-Reactivity & Interference

Adenovirus 2	Dengue virus 4	HIV-1	Influenza A virus	Zika virus	Staphylococcus epidermidis
Adenovirus 5	Epstein-Barr virus	HIV-2	Parvovirus B19	Candida albicans	
Cytomegalovirus	Hepatitis A Virus	Human papillomavirus 16	Rubella virus	Propionibacterium acnes	
Dengue virus 1	Hepatitis B Virus	Human papillomavirus 18	St. Louis encephalitis virus	Staphylococcus aureus	
Dengue virus 2	Human herpes virus 6B	Human T-cell lymphotropic virus-type 1	Yellow Fever	Neisseria gonorrhoeae	
Dengue virus 3	Human herpes virus 8	Human T-cell lymphotropic virus-type 2	West Nile	Chalymidia trachomatis	

Non-Target Organisms	Average conc. (Log10 IU/mL)	Bias* (Log10 IU/mL)
Pool 1: Cytomegalovirus, Epstein-Barr virus, Human Herpesvirus 6B, Human Herpesvirus 8, Rubella	1.71	0.31
Pool 3: Hepatitis A, Hepatitis B, Zika virus, Yellow Fever, Human Immunodeficiency virus 1	0.99	0.41
Pool 6: Candida albicans, Chlamydia trachomatis, Herpes Simplex virus 1, Herpes Simplex virus 2, Staphylococcus aureus, Staphylococcus epidermidis	1.44	0.04

Non-Target Organisms	Average conc. (Log10 IU/mL)	Bias* (Log10 IU/mL)
Adenovirus 2	1.75	0.35
Adenovirus 5	1.09	0.31
Dengue 4	1.61	0.21
Parvo B19	1.76	0.36
Dengue 2	1.55	0.15
Dengue 3	1.67	0.27
St Louis Encephalitis	1.64	0.25
Dengue 1	1.77	0.37
Human T-lymphotropic virus 1	1.59	0.19
Human T-lymphotropic virus 2	1.55	0.15
Influenza A	1.6	0.20
Human Immunodeficiency virus 2	1.54	0.15
Human papillomavirus 16	1.5	0.10
Human papillomavirus 18	1.53	0.13
Neisseria Gonorrhoea	1.64	0.24
Propionibacterium acnes	1.41	0.01
West Nile	1.65	0.25

HCV Interfering Substances – Commensal Organisms. The same high titers of the same 31 non-target organisms (pooled or individual) tested. The NeuMoDx HCV Test had minimal deviation of quantitation from HCV control samples, with no significant interference.

▲ **HCV Analytical Specificity – Cross-Reactivity.** The NeuMoDx HCV Test had no cross-reactivity with 31 non-target (or phylogenetically similar) organisms found in blood/plasma specimens – demonstrated 100% analytical specificity.

Endogenous	Average conc. (Log10 IU/mL)	Bias* (Log10 IU/mL)
Bilirubin	1.47	0.14
Protein (albumin)	1.47	0.14
Hemoglobin	1.61	0.28
Triglycerides	1.31	0.02
Disease State	**Average conc. (Log10 IU/mL)**	**Bias* (Log10 IU/mL)**
Systemic Lupus Erythematosus (SLE)	1.29	0.06
Antinuclear Antibody (ANA)	1.53	0.18
Rheumatoid Arthritis (RA)	1.39	0.04
Rheumatoid Factor (RF)	1.43	0.08
Non-Alcoholic Steatohepatitis (NASH)	1.32	0.03
HBV Antibodies	1.45	0.10
HCV Antibodies	1.43	0.08
Alcoholic cirrhosis (AC)	1.29	0.06

Non-Target Organisms	Average conc. (Log10 IU/mL)	Bias* (Log10 IU/mL)
Pool 1: Sofosbuvir, Ledipasvir, Velpatasivr, Clarithromycin, Interferon alfa-2a	1.48	0.15
Pool 2: Paritaprevir, Ombitasvir, Ritonavir, Abacavir sulfate, Ribavirin	1.4	0.07
Pool 3: Grazoprevir, Elbasvir, Tenofovir disoproxil, Lamivudine, Valganciclovir	1.4	0.07
Pool 4: Efavirenz, Lopinavir, Azithromycin, Dolutegravir, Simeprevir	1.51	0.18
Pool 5: Emtricitabine, Raltegravir, Amoxicillin, Rilpivirine, Dasabuvir, Glecaprevir	1.4	0.07

HCV Interfering Substances – Endogenous & Exogenous Substances. Twelve (12) different endogenous/disease state specimens & 26 exogenous substances (drugs) tested. The NeuMoDx HCV Test had minimal deviation of quantitation from HCV control samples, with no significant interference.

Precision

The Within Lab Precision of the NeuMoDx HCV test was determined by testing a 7 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 Log10 IU/mL.

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 ≤ 0.26 Log10 IU/mL.

NeuMoDx HCV Test Within Lab Precision							
Panel Member	Target Conc. (Log10 IU/mL)	Mean Conc. (Log10 IU/mL)	N	Within System SD	Within Day SD	Within Run SD	(Overall) Within Lab SD
1	6.0	5.95	216	0.17	0.13	0.10	0.17
2	5.0	4.87	216	0.20	0.14	0.12	0.20
3	3.0	2.89	217	0.19	0.17	0.17	0.19
4	4.4	4.45	216	0.12	0.10	0.08	0.13
5	3.4	3.45	216	0.12	0.12	0.11	0.13
6	2.4	2.41	220	0.17	0.15	0.15	0.17
7	1.4	1.40	217	0.26	0.25	0.25	0.24

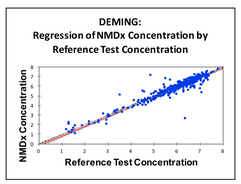
Method Correlation

The quantitative performance of the NeuMoDx HCV Test was assessed against 4 CE/FDA approved comparator tests by testing clinical specimens from HCV infected patients.

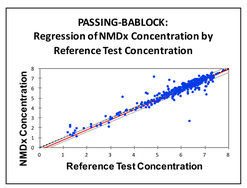
A total of 646 clinical plasma and serum specimens within the linear range were used to generate the linear regression.

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 with Deming and Passing-Bablok linear regression tests.



Deming Analysis
Intercept
-0.16 95%CI (-0.37,0.06)
Slope Coefficient
1.00 95%CI (0.966,1.03)



Passing-Bablok Analysis
Intercept
-0.28 95%CI (-0.43,-0.14)
Slope Coefficient
1.02 95%CI (0.99,1.05)

TRUTH TABLE**	REFERENCE TESTS POSITIVE	REFERENCE TESTS NEGATIVE	Total
NMDx TEST POSITIVE	360	14	374
NMDx TEST NEGATIVE	1	271	272
Total	361	285	646
POSITIVE AGREEMENT = 99.7% 95% CI (98.5% - 100%)			
NEGATIVE AGREEMENT = 95.1% 95% CI (91.7% - 97.2%)			

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

CONCLUSIONS & ACKNOWLEDGMENTS

The performance data presented here clearly demonstrate that the NeuMoDx HCV Test is an extremely easy to use, rapid, automated test for the sensitive and accurate monitoring of HCV viral load. 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.

NeuMoDx HCV Test is not available for sale