QUANTITATIVE DETECTION OF HCV USING THE NEUMODX MOLECULAR SYSTEM

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BACKGROUND

Determining hepatitis C virus (HCV) RNA levels in plasma and 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 Quant Assay is a fully automated, "sample to result" *in-vitro* diagnostic test for the accurate quantitation of HCV RNA directly from plasma and serum specimens. The NeuMoDx HCV Quant Assay incorporates reagents and disposables designed to be room temperature stable and remain on-board the system to provide a seamless, on-demand testing workflow in a truly "random access" manner on the NeuMoDx Molecular systems.

METHODS

The NeuMoDx HCV Quant Assay incorporates a universal nucleic acid isolation chemistry combined with a sensitive quantitative RT-PCR assay to deliver highly accurate results with a rapid turnaround time. The objective of this study was to characterize various performance metrics in support of CE-IVD certification - analytical sensitivity & specificity, linearity, precision, inclusivity, turnaround time, as well as quantitative correlation to FDA-approved/CE-IVD-certified reference tests using clinical specimens.

RESULTS

The NeuMoDx™ HCV Quant Assay has a sensitivity of 7.5 IU/mL and 8.0 IU/mL in plasma and serum respectively with LLoQ of 7.7 IU/mL and 8.4 IU/mL using the 5th WHO International Standard for HCV. The NeuMoDx HCV Quant Assay demonstrated excellent linearity across a 7-log dynamic range (R2>0.99) as well as precision across 12 days with maximum overall deviation of 0.26 Log10 IU/mL at lowest level tested. The NeuMoDx HCV Quant Assay showed equivalent detection across all relevant HCV genotypes and a time to first result of ~80 min. No cross-reactivity or interference was observed against a panel of 31 pathogens and common interfering agents tested. The method correlation study performed using 646 clinical remnant plasma and serum specimens across two different NeuMoDx systems [N288 and N96 Systems] demonstrated excellent concordance with the respective reference test as indicated by a slope of 1.0 and intercept of less than 0.3.

NeuMoDx Molecular System Streamlined Testing

WORKFLOW

EASE OF USE: LOAD SPECIMEN(S), WALK AWAY AND RESULTS IN 60-75 MIN

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:** ~80 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.

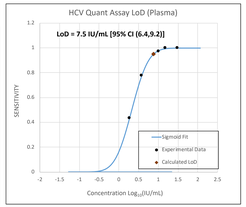
HCV Analytical Sensitivity (LoD & LLoQ)

PLASMA

The Limit of Detection of the NeuMoDx™ HCV Quant Assay was determined with pooled HCV negative plasma spiked with 5th WHO International Standard at seven different concentrations 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 Quant Assay 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	107	99%	
10	1.00	108	102	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 Quant Assay. 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) in plasma.

NeuMoDx HCV Quant Assay 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	

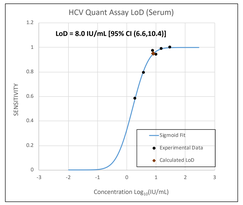
Lower limit of quantitation (LLoQ) of NeuMoDx HCV Quant Assay. The lowest target level detected at a rate > 95% AND with TAE (bias+2*SD) ≤ 1.0 was used to determine the LLoQ. The LLoQ of the NeuMoDx HCV Quant Assay was determined to be 7.7 IU/mL in plasma.

SERUM

The Limit of Detection of the NeuMoDx HCV Quant Assay was determined with pooled HCV negative plasma spiked with AcroMetrix High Control HCV (traceable to the WHO 5th IS) at seven different concentrations 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 Quant Assay 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 Quant Assay. Probit analysis from the data in the above table was used to determine the LoD of the HCV target to be 8.0 IU/mL with a 95% CI of (6.6,10.4) in serum.

NeuMoDx HCV Quant Assay 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 Quant Assay. 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 Quant Assay was determined to be 8.4 IU/mL in serum.

GENOTYPE SENSITIVITY

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

HCV Genotype	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 Quant Assay accurately detected all six relevant genotypes of HCV in negative plasma with LoD at or under the WHO 5th International Standard LoD.

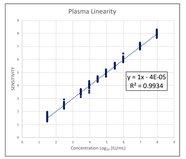
HCV Linearity

PLASMA

The linearity of the NeuMoDx™ HCV Quant Assay was determined by diluting either Acrometrix HCV Positive Control or Asuragen HCV Armored RNA® in pooled HCV negative plasma to create a panel spanning HCV concentrations ranging from 8.0 Log10 IU/mL to 1.5 Log10 IU/mL.

Data from this study showed that the NeuMoDx HCV Quant Assay demonstrated excellent linearity across the 8 log panel.

NeuMoDx HCV Quant Assay Linearity in Plasma				
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 Quant Assay. The NeuMoDx HCV Quant Assay is linear over 8 Log_{10} units.

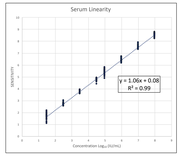
Linear range of the NeuMoDx HCV Quant Assay. The NeuMoDx HCV Quant Assay shows excellent correlation over 8 Log_{10} units.

SERUM

The linearity of the NeuMoDx HCV Quant Assay 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 Quant Assay demonstrated excellent linearity across the 8 logs.

NeuMoDx HCV Quant Assay Linearity in Serum				
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 Quant Assay. The NeuMoDx HCV Quant Assay is linear over 8 Log_{10} units.

Linear range of the NeuMoDx HCV Quant Assay. The NeuMoDx HCV Quant Assay shows excellent correlation over 8 Log_{10} units.

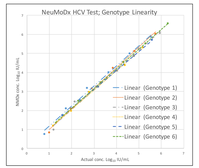
GENOTYPE LINEARITY

The linearity of the NeuMoDx HCV Quant Assay 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 Quant Assay demonstrated excellent linearity across the 6 log panel.

Genotype	Linearity Equation*	R^2
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 Quant Assay. Confirmed with 6 genotypes of HCV.



Within Lab Precision

The NeuMoDx™ HCV Quant Assay 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 Quant Assay 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

Cross-Reactivity and Interference

Non-Target Organisms	Exogenous Substances (Medications)
Adenovirus 2	Ledipasvir
Adenovirus 5	Velpatasvir
Cytomegalovirus	Sofosbuvir
Dengue virus 1	Interferon alfa-2a
Dengue virus 2	Ombitasvir
Dengue virus 3	Ritonavir
Dengue virus 4	Abacavir sulfate
Epstein-Barr virus	Clarithromycin
Hepatitis A virus	Paritaprevir
Hepatitis B virus	Grazoprevir
Human herpes virus 6B	Efavirenz
Human herpes virus 8	Dasabuvir
HIV-1	Elbasvir
HIV-2	Tenofovir disoproxil
Human papillomavirus 16	Lamivudine
Human papillomavirus 18	Valganciclovir
Human T-cell lymphotropic virus-type 1	Lopinavir
Human T-cell lymphotropic virus-type 2	Azithromycin
Influenza A virus	Dolutegravir
Parvovirus B19	Simeprevir
Rubella virus	Emtricitabine
St. Louis Encephalitis virus	Raltegravir
Yellow Fever virus	Rilpivirine
West Nile virus	Amoxicillin
Zika virus	Glecaprevir
Candida albicans	**Disease State Samples**
Propionibacterium acnes	Systemic Lupus Erythematosus (SLE)
Staphylococcus aureus	Antinuclear Antibody (ANA)
Neisseria gonorrhoeae	Rheumatoid Arthritis (RA)
Chlamydia trachomatis	Rheumatoid Factor (RF)
Staphylococcus epidermidis	
Endogenous Substances	**Non-Alcoholic Steatohepatitis (NASH)**
Bilirubin	HBV Antibodies
Protein (albumin)	Alcoholic cirrhosis (AC)
Hemoglobin	
Triglycerides	

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

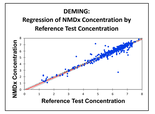
Method Correlation

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

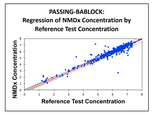
A total of 646 clinical plasma and serum specimens within the linear range were used to generate the linear regression.

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

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

***Excludes NMDx positive samples below LLoQ and below 1.4 Log10 IU/mL*

CONCLUSIONS

The NeuMoDx™ HCV Quant Assay is an extremely easy to use, rapid, automated test for the sensitive and accurate monitoring of HCV viral load.

ACKNOWLEDGMENTS

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