QUANTITATIVE DETECTION OF HEPATITIS B VIRUS USING AN INTEGRATED MOLECULAR DIAGNOSTIC SYSTEM

TUESDAY-14

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BACKGROUND

Quantitation of hepatitis B virus (HBV) DNA titers in infected patients is essential to monitor disease progression and efficacy of antiviral therapies, as well as to detect drug resistant mutants and identify relapse upon discontinuation of antiviral therapy. The NeuMoDx HBV Test is a fully automated in-vitro diagnostic test offered on the NeuMoDx 288 Molecular System which combines universal nucleic acid isolation chemistry with sensitive real-time PCR detection of HBV particles. Upon loading the samples, the System automatically extracts, isolates, and purifies DNA using proprietary reagents and a microfluidic cartridge. The System then automatically uses the purified DNA solution to rehydrate the PCR assay reagents and initiate real-time PCR to deliver rapid and accurate quantitative results. The objective of this study was to test and report performance of the NeuMoDx HBV Test against key performance metrics.

METHODS

Analytical studies were performed to determine sensitivity, limits of quantitation, linearity, inclusivity, effect of cross-reacting or interfering agents, within-lab precision, reproducibility across reagent lots and turnaround time of the NeuMoDx HBV Test. Analytical sensitivity was determined using the 4th WHO International Standard for HBV. The Limit of Detection (LoD) and Lower Limit of Quantitation (LLoQ) along with linearity parameters were also assessed across the different HBV genotypes. Specificity was confirmed by screening 32 phylogenetically similar or cohabitating organisms while 38 different endogenous and exogenous substances were tested for potentially interfering effect. Within-Lab Precision was assessed over 12 days on three Systems and lot to lot reproducibility was determined across three lots of reagents. An in-house method correlation study was also performed to assess quantitative concordance with reference tests.

RESULTS

The NeuMoDx HBV Test demonstrated a LoD of 6.2 IU/mL and a LLoQ of 7.6 IU/mL across all relevant genotypes. The test demonstrated excellent linearity across an 8 Log dynamic range (9.02-1.02 Log_{10} IU/mL; R^2=0.998) with an accuracy of ±0.22 Log_{10} IU/mL across the entire range and the Upper Limit of Detection (ULoQ) was determined to be 9.02 Log_{10} IU/mL. The NeuMoDx HBV Test showed no crossreactivity with the 32 organisms tested and demonstrated robustness in the presence of endogenous and exogenous interfering agents. An overall standard deviation of <0.25 Log_{10} IU/mL was obtained for both within-lab precision and lot to lot reproducibility studies. The turnaround time of NeuMoDx HBV Test was ~60 minutes. Method comparison studies performed on 97 clinical samples confirmed excellent linear correlation (R^2>0.97) and minimal bias (0.20 Log_{10} IU/mL) relative to a reference test. Finally, 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

Ease of use: Load specimen(s), walk away and results in ~60 min

LOAD SPECIMENS

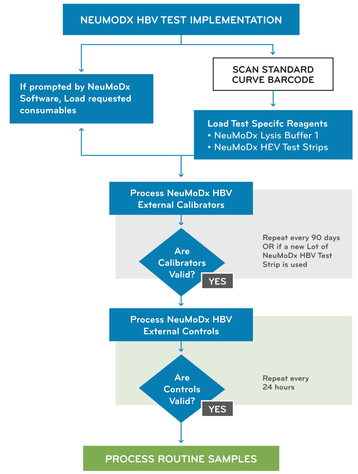
INITIATE TESTING WITH 2 ON-SCREEN SELECTIONS

RESULT IN ~60 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 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

NeuMoDx Quantitative Test Workflow



NeuMoDx HBV External Calibrators and NeuMoDx HBV External Controls are traceable to the 4th WHO International Standard for HBV DNA.

Analytical Sensitivity

NEUMODX HBV TEST LIMIT OF DETECTION AND LOWER LIMIT OF QUANTITATION

Summary LoD Study - 4th WHO IS (Genotype A)				
HBV Conc. (IU/mL)	HBV Conc. (Log_{10} IU/mL)	Number of Valid Tests	Number of Positives	Detection Rate
20	1.3	108	108	100%
10	1	108	107	99%
5	0.7	108	98	91%
2.5	0.4	108	97	90%
1.25	0.1	108	73	68%
0	0	108	0	0%

Summary LLoQ Study - 4th WHO IS (Genotype A)						
HBV Conc. (IU/mL)	HBV Conc. (Log_{10} IU/mL)	Detection Rate	NMDx Average Conc. (Log_{10} IU/mL)	SD	Bias	TAE
20	1.30	100%	1.29	0.23	0.16	0.63
10	1.00	99%	1.07	0.25	0.20	0.71
5	0.70	91%	0.89	0.35	0.34	1.04
2.5	0.40	90%	0.75	0.44	0.51	1.39
1.25	0.10	68%	0.73	0.41	0.68	1.50

GENOTYPE	LoD (IU/mL)	LLoQ (IU/mL)
Genotype A	5.2	5.2
Genotype B	6.2	6.2
Genotype C	3.5	6.2
Genotype D	5.2	5.7
Genotype E	3.5	3.5
Genotype F	5.1	6.2
Genotype G	3.5	3.5
Genotype H	5.2	7.6

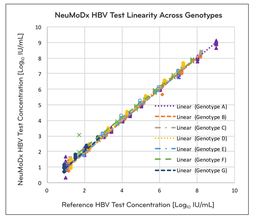
LoD using 4th International Standard was determined to be 5.2 IU/mL (0.71 Log_{10} IU/mL) Across Genotypes, LoD was determined to be 6.2 IU/mL (0.79 Log_{10} IU/mL).

LLoQ using 4th International Standard was determined to be 5.2 IU/mL (0.71 Log_{10} IU/mL) Across Genotypes, LLoQ was determined to be 7.6 IU/mL (0.88 Log_{10} IU/mL).

Linearity

NEUMODX HBV TEST LINEARITY AND UPPER LIMIT OF QUANTITATION

Summary Linearity Study - HBV Positive Sample (Genotype A)						
Target Conc. (Log_{10} IU/mL)	Mean Conc. (Log_{10} IU/mL)	N	Standard Deviation	Bias	Predicted Linear Fit	Deviation from Non-Linear Fit
9.02	8.99	36	0.08	0.06	9.02	-0.04
8.02	8.05	36	0.07	0.05	8.02	0.03
7.02	7.05	36	0.07	0.06	7.02	0.04
6.02	6.05	36	0.05	0.05	6.02	0.03
5.02	5.04	36	0.05	0.04	5.02	0.00
4.45	4.43	36	0.07	0.05	4.45	-0.01
4.02	3.99	36	0.09	0.05	4.02	-0.02
3.45	3.41	36	0.07	0.06	3.45	-0.03
3.02	3.00	36	0.04	0.04	3.02	-0.03
2.02	1.99	36	0.11	0.09	2.02	-0.01
1.02	1.09	36	0.29	0.23	1.02	0.06



Genotype	Linearity Equation*	R^2
A	y = 1x + 1E-06	0.9977
B	y = 1.0129x - 0.0964	0.9975
C	y = 1.0250x - 0.0898	0.998
D	y = 1.0379x - 0.0896	0.9975
E	y = 1.0182x - 0.096	0.9956
F	y = 0.9736x + 0.276	0.9906
G	y = 1.0547x - 0.0835	0.9813

*where x is Expected Quantitation and y is NeuMoDx HBV Test Quantitation expressed in Log_{10} IU/mL. Genotype H was excluded from the linearity study due to low stock concentration of 741 IU/mL. Sources of HBV Genotypes: Access Biologicals, Discovery Life Sciences, PEI, Qnostics.

Excellent Linearity Demonstrated Across HBV Genotypes.

Precision

Panel Member	HBV Conc. (Log_{10} IU/mL)	NMDx Mean Conc. (Log_{10} IU/mL)	N	Bias	Within LOT SD	Across LOTS SD	Overall SD
Genotype A	8.02	7.99	36	0.03	0.15	0.09	0.17
	6.02	5.96	36	0.06	0.17	0.06	0.18
	4.02	3.90	36	0.12	0.14	0.09	0.17
	2.02	1.92	36	0.10	0.21	0.12	0.24
Genotype C	6.27	6.32	36	0.05	0.06	0.08	0.10
	4.27	4.31	36	0.04	0.22	0.09	0.24
	3.27	3.30	36	0.03	0.20	0.07	0.21
	2.27	2.32	36	0.05	0.13	0.13	0.18

Study performed over 12 days on 3 NeuMoDx Molecular Systems and using one lot of NeuMoDx reagents. Maximum Bias of 0.23 Log_{10} IU/mL and maximum overall Standard Deviation of 0.22 Log_{10} IU/mL.

Lot to Lot Reproducibility

Panel Member	HBV Conc. (Log_{10} IU/mL)	NMDx Mean Conc. (Log_{10} IU/mL)	N	Bias	Within Run SD	Within Day SD	Within System SD	Overall SD
Genotype A	7.75	7.89	36	0.14	0.06	0.07	0.07	0.07
	5.75	5.83	36	0.11	0.07	0.10	0.10	0.10
	3.75	3.70	36	0.11	0.11	0.13	0.15	0.15
	1.75	1.54	36	0.23	0.16	0.22	0.22	0.22
Genotype C	6.27	6.23	36	0.10	0.08	0.09	0.10	0.10
	4.27	4.18	36	0.08	0.08	0.10	0.10	0.10
	3.27	3.14	36	0.09	0.08	0.12	0.12	0.12
	2.27	2.08	36	0.12	0.12	0.15	0.16	0.16

Study performed using 3 different lots of NeuMoDx HBV Test Strips, NeuMoDx Lysis Buffer 1, and NeuMoDx Extraction Plates. Maximum Bias of 0.12 Log_{10} IU/mL and maximum overall Standard Deviation of 0.24 Log_{10} IU/mL.

Cross-Reactivity

Adenovirus 2	Dengue V1	Hepatitis A	HPV 16	Ilheus (ILHV)	Yellow Fever
Adenovirus 5	Dengue V2	Hepatitis C	HPV 18	Influenza A	Zika Virus
Banzi Virus	Dengue V3	Human Herpesvirus 6a	HSV1	Parvo B19	
BK Virus	Dengue V4	Human Herpesvirus 8	HSV 2	Rubella,	
Cytomegalovirus	Epstein Barr Virus	HIV 1	HTLV 1	St. Louis Encephalitis	
VZV	Vaccinia Virus	HIV 2	HTLV 2	West Nile Virus	

No Cross-Reactivity Observed, NeuMoDx HBV Test Demonstrated 100% Analytical Specificity.

Interfering Substances

Non-Target Organisms	Average Conc. (Log_{10} IU/mL)	Bias* (Log_{10} IU/mL)
Pool 1: BK Virus, Cytomegalovirus, Epstein Barr Virus, Human Herpesvirus 6a, Human herpesvirus 8	3.51	0.10
Pool 2: Adenovirus 2, Adenovirus 5, Dengue V2, Dengue V3, Dengue V4	3.38	0.22
Pool 3: Parvo B19, HTLV 1, HTLV 2, Ilheus (ILHV), Yellow Fever, Zika Virus	3.62	0.06
Pool 4: HPV 16, HPV 18, HSV 1, HSV 2, Dengue V1	3.57	0.04
Pool 5: St. Louis Enceph, VZV, Vaccinia Virus, West Nile Virus	3.57	0.03

Non-Target Organisms	Average Conc. (Log_{10} IU/mL)	Bias* (Log_{10} IU/mL)
HAV	3.58	0.05
Banzi virus	3.41	0.19
HIV-1	3.47	0.15
HIV-2	3.56	0.05
Rubella	3.16	0.44
Influenza A	3.60	0.03
HCV	3.58	0.04

*Concentration of HBV controls: ~3.6 Log_{10} IU/mL

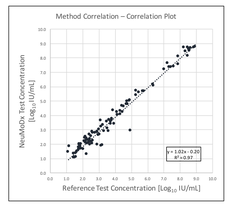
Endogenous	Average Conc. (Log_{10} IU/mL)	Bias* (Log_{10} IU/mL)
Hemoglobin	3.5	0.2
Triglycerides	3.51	0.09
Bilirubin	3.56	0.13
Albumin	3.51	0.17
Disease State	**Average Conc. (Log_{10} IU/mL)**	**Bias* (Log_{10} IU/mL)**
Antinuclear Antibody (ANA)	3.61	0.1
Systemic Lupus Erythematosus (SLE)	3.63	0.1
Rheumatoid Arthritis (RA)	3.57	0.09
HCV Antibodies	3.58	0.07
HBV Antibodies	3.64	0.11
Alcoholic cirrhosis	3.68	0.15
Rheumatoid Factor (RF)	3.63	0.1
Non-Alcoholic Steatohepatitis (NASH)	3.49	0.06

Exogenous (Drugs)	Average Conc. (Log_{10} IU/mL)	Bias* (Log_{10} IU/mL)
Pool 1: Zidovudine (ZDV), Saquinavir, Ritonavir, Clarithromycin, Interferon alfa-2a, Interferon alfa-2b	3.58	0.08
Pool 2: Abacavir sulfate, Amprenavir, Ribavirin, Entecavir, Fluoxetine, Valacyclovir Hydrochloride	3.56	0.04
Pool 3: Tenofovir disoproxil, Lamivudine, Ganciclovir, Valganciclovir, Nevirapine	3.59	0.06
Pool 4: Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin, Paroxetine	3.6	0.07
Pool 5: Adefovir (dipivoxil), Azithromycin, Indinavir sulfate, Sertaline	3.56	0.19

*Concentration of HBV controls: ~3.7 Log_{10} IU/mL

None of the Commensal Organisms or Endogenous and Exogenous Substances Tested showed Any Significant Adverse Effect on the Quantitation Efficacy of the NeuMoDx HBV Test.

Method Correlation



Correlation Study Plot compiles the data of 97 HBV Positive Samples for which quantitative values were obtained by both the NeuMoDx HBV Test and the reference test. Excellent correlation and minimal bias of -0.20 Log_{10} IU/mL.

TRUTH TABLE	REFERENCE TESTS POSITIVE	REFERENCE TESTS NEGATIVE	Total
NMDx TEST POSITIVE	99	5	104
NMDx TEST NEGATIVE	0	196	196
Total	99	201	300
SENSITIVITY = 100% 95% CI (95.5% - 100%)			
SPECIFICITY = 97.5% 95% CI (94.0% - 99.1%)			

A total of 300 plasma samples, previously processed using one of 3 different CE/FDA-cleared HBV Tests, were included in this study. Excellent concordance to reference tests demonstrated.

CONCLUSION

The NeuMoDx HBV Test offers a rapid and high throughput technology in an on-demand, sample-to-result testing format. These features coupled with excellent sensitivity and accuracy performance metrics make the NeuMoDx HBV Test an outstanding solution for viral load monitoring required for effective patient management of blood borne virus infections.

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