READY TO USE MOLECULAR DIAGNOSTIC REAGENTS WITH LONG TERM AMBIENT AND ON-BOARD STORAGE FOR FULLY AUTOMATED SYSTEMS

Betty Wu, Elizabeth Craig, Crystal Nguyen, Hui Lin Lee, Lijie Gong, Will Marshall, Mark Olson, and S Brahmasandra, NeuMoDx Molecular, Inc, Ann Arbor, MI

BACKGROUND

Reagents used for molecular diagnostics are typically stored frozen or at least refrigerated to maintain viability. The proprietary NeuDry process developed by NeuMoDx offers a simple, low cost, and rapid process using a conventional conveyer oven to produce ready to use, ambient condition stable reagents capable of providing long open-shelf life for active reagents used in molecular diagnostic testing. The performance of reagents produced using the NeuDry process across wide variety of molecular diagnostic applications is presented here.

METHODS

Three types of reagents formulated and optimized for both long term storage and in-use shelf life were produced using the NeuDry process - 1) **Nucleic acid isolation** reagents including NeuMag™ affinity particles, lytic enzyme, and encapsulated DNA/RNA process controls, 2) **PCR Master mix**, and 3) **RT-PCR Master mix**. Performance of the NeuDry reagents was initially evaluated against the liquid version of these reagents. Efficacy of the reagents was further evaluated by testing across a variety of clinical matrices encompassing both DNA and RNA targets. Long-term stability and in-use shelf life was determined via accelerated and real time stability studies on fully automated NeuMoDx Molecular System.

DRYING PROCESS

PCR OR RT-PCR FORMULATION
NUCLEIC ACID EXTRACTION FORMULATION

DISPENSE

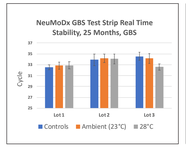
DRY, 15-20 MIN

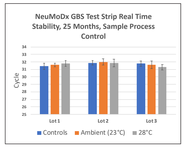
PACKAGE

RESULTS

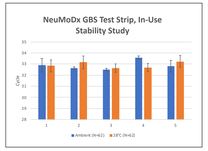
NeuDry extraction reagents demonstrated >24 months shelf life and 63 days in-use stability in real time stability studies, with excellent sensitivity in a variety of matrices, such as plasma, serum, whole blood, urine and swab specimens. The NeuDry PCR/RT-PCR reagents, for both qualitative and quantitative assays, provided excellent amplification relative to liquid reagents as measured by Ct shift (<1 cycle) and relative fluorescence increase (>80%). The PCR Master Mix formulated for the detection of GBS provided 25 months shelf life when stored at 18-28°C and up to 63 days in-use shelf life in real time stability studies. For both DNA and RNA viral quantitative assays, formulations for detecting HBV, CMV and HCV, have demonstrated over 12 months stability and up to 63 days in-use shelf life with no loss of activity in real time and/or accelerated stability studies. The NeuDry process has also proven to be easily applicable to master mixes using different *Taq* polymerases without substantial re-formulation. The entire drying process takes less than 30 min resulting in over 10,000 tests produced in a single 8-hour shift with two operators and one conveyor oven. In addition, the continuous workflow enables significantly easier automation (& reduced cost) compared to batch processes such as lyophilization process.

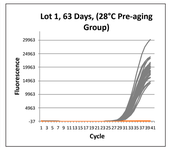
NEUMODX GBS TEST STRIP: REAL TIME STABILITY STUDY, 25 MONTHS





- NeuMoDx Group B Strep (GBS) Assay is a qualitative in vitro diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA.
- Three lots of GBS Test Strips were stored at ambient temperature (~ 23°C) and 28°C at an average 40-45% humidity for 25 months.
- Stability of the NeuMoDx GBS Test Strips by testing aged strips on a NeuMoDx System using GBS spiked into Lim Broth at concentration of 1000 CFU/mL.
- 27 replicates of GBS positive samples and 3 negatives tested for each lot of NeuMoDx GBS Test Strip per storage condition.
- Aged group performed equally well as unaged Control group based on positive detection rate of 100% demonstrated for both storage conditions.

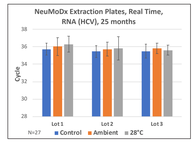


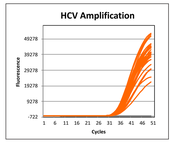


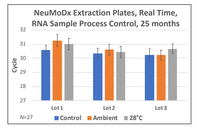
Pre-Aging	Ambient (N=62)			28°C (N=62)		
In-Use, Days	Avg	SD	% Pos	Avg Ct	SD	% Pos
0	32.9	0.59	100%	32.8	0.55	100%
15	32.6	0.13	100%	33.1	0.60	100%
32	32.5	0.11	100%	32.6	0.36	100%
43	33.6	0.13	100%	32.7	0.37	100%
63	32.8	0.52	100%	33.2	0.53	100%
ΔCt	-0.1			0.4		

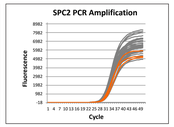
- Two lots NeuMoDx GBS Test Strips were pre-aged as part of real time stability study for 12 months.
- The test strips were removed from the packaging, and stored at "in-use" conditions - average temperature at 28°C & average of 40-45% humidity - for up to 63 days
- Activity of the strips accessed by testing of the aged strips on a NeuMoDx System using GBS spiked into Lim Broth at concentration of 1000 CFU/mL.
- 31 replicates of GBS positive samples and 3 negatives tested for each lot of GBS Test Strip per pre-storage condition.
- The test strips aged 63 days at "in-use" conditions performed equivalently to day 0 based on the positive detection rate and ΔCt from time 0.

NEUMODX EXTRACTION PLATE STABILITY – REAL TIME, 25 MONTHS



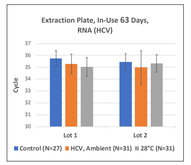


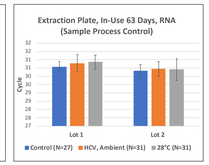




- NeuMoDx Extraction Plate contains all the reagents required for DNA and RNA extraction, such as lytic enzymes and Neu magnetic beads, as well as DNA and RNA Sample Process Controls.
- Three lots of Extraction Plates stored at ambient temperature (~ 23°C) and 28°C with average 40-45% humidity for 25 months
- Stability of the Extraction Plates was evaluated by testing of the aged Extraction Plates on the NeuMoDx System using NeuMoDx HCV Assay (GBS Assay Data previously presented in the figures on the left)
- Stability of the Extraction Plates for RNA assays was evaluated with a model RNA assay, NeuMoDx HCV Assay. HCV viral particles were spiked into human plasma at concentration of 60 IU/mL (27 replicates) and processed on a NeuMoDx Molecular System using aged and unaged extraction plates.
- Aged group performed equally well based on the 100% amplification of both HCV target and the RNA Sample Process Control with low SD of Ct values.

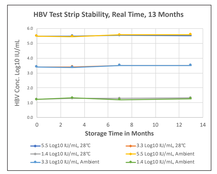
NEUMODX EXTRACTION PLATE STABILITY – IN-USE, 63 DAYS AT 25 MONTH

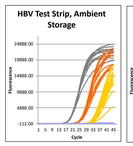


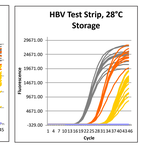


- Two lots NeuMoDx Extraction Plates were pre-aged as part of real time stability study for 25 months.
- The Extraction Plates were removed from the packaging, and stored at "in-use" conditions - average temperature at 31°C & average of 40-45% humidity - for up to 63 days
- Stability of the Extraction Plates was evaluated by testing of the aged Extraction Plates on the NeuMoDx System using NeuMoDx HCV Assay (GBS Assay Data previously presented in the figures on the left)
- HCV viral particles were spiked into human plasma at concentration of 60 IU/mL and processed on a NeuMoDx Molecular System.
- Aged group performed equally well based on the 100% amplification of both HCV target and RNA sample process control with low SD of Ct values.

NEUMODX HBV QUANT ASSAY TEST STRIP: REAL TIME STABILITY - 13 MONTHS



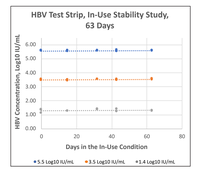


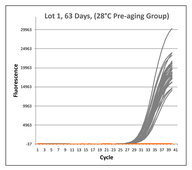


	NeuMoDx Test Strips Shelf Life, 13 Months										
HBV Conc [Log10 IU/mL]	Storage Condition	Time 0		3 Months		7 Months		13 Months		Slope	% Drift	ΔTime 0 at 13 M
		Avg	SD	Avg	SD	Avg	SD	Avg	SD			
	Ambient	5.48	0.01	5.49	0.02	5.53	0.07	5.53	0.04	0.0038	0.38%	-0.02
	28°C	5.48	0.01	5.46	0.05	5.57	0.03	5.58	0.01	0.0094	0.94%	0.10
	Ambient	3.41	0.05	3.42	0.05	3.52	0.03	3.52	0.06	0.0100	1.00%	-0.05
	28°C	3.41	0.05	3.42	0.05	3.52	0.02	3.52	0.04	0.0098	0.98%	0.11
	Ambient	1.22	0.02	1.28	0.05	1.28	0.04	1.31	0.07	0.0065	0.65%	-0.01
	28°C	1.22	0.02	1.28	0.03	1.28	0.04	1.31	0.13	0.0015	0.15%	0.09

- Three lots of NeuMoDx HBV Quant Assay Test Strips were stored at ambient (~ 23°C) and 28°C with average 40-45% humidity for 13 months.
- Activity of the test strips was evaluated by testing the aged test strips on a NeuMoDx System using human plasma spiked with HBV at concentrations of 5.5, 3.5 (10 replicates at each level) and 1.4 log10 IU/mL (20 replicates) per lot for each storage condition
- Linear regression analysis demonstrated minimal drift after 13 months - ≤ 1%, in quantification of HBV after storage for 13 months.

NEUMODX HBV QUANT ASSAY TEST STRIP: IN-USE STABILITY – 63 DAYS

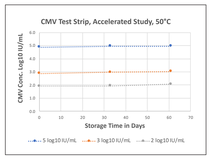


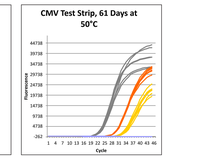


Expected HBV Conc, [Log10 IU/mL]	D0		D15		D32		D43		D63		Δ fr D0 [Log10 IU/mL]	% Drift
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD		
5.63	5.58	0.02	5.56	0.01	5.59	0.01	5.59	0.09	5.60	0.03	0.02	0.03%
3.63	3.51	0.01	3.47	0.01	3.54	0.00	3.52	0.05	3.55	0.05	0.03	0.07%
1.40	1.28	0.04	1.27	0.13	1.41	0.04	1.33	0.11	1.28	0.03	0.00	0.06%

- In-Use stability study of NeuMoDx HBV Test Strips. HBV Test Strips were first aged at ambient or 28°C as part of the Real Time Stability study. Two lots of HBV Test Strips were removed from their primary storage condition and stored at In-Use condition (31°C with average 40-45% humidity) up to 61 days.
- The activity of the strips was evaluated by testing the aged strips on the NeuMoDx System using human plasma spiked with HBV at concentrations of 5.5, 3.5 (10 replicates at each level) and 1.4 log10 IU/mL (20 replicates) per lot for each storage condition.
- There is minimum drift, ≤ 0.07%, in quantification of HBV up to 61 days at the In-Use condition for both pre-aging groups, ambient or 28°C. The data from the 28 °C pre-aging group are presented here.

NEUMODX CMV QUANT ASSAY TEST STRIP: ACCELERATED STUDY

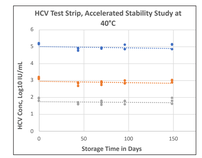


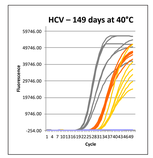


CMV Conc	D0					33 Days (7 Months Equivalent)					61 Days (13 Months Equivalent)					% Drift	Avg Δ fr D0
	Lot 1	Lot 2	Lot 3	3 Lots Avg	SD	Lot 1	Lot 2	Lot 3	3 Lots Avg	SD	Lot 1	Lot 2	Lot 3	3 Lots Avg	SD		
5.00 log10 IU/mL	4.87	4.93	4.84	4.88	0.05	4.89	5.03	4.95	4.96	0.07	4.92	5.05	4.93	4.97	0.07	0.14	0.09
3.00 log10 IU/mL	2.90	2.86	2.89	2.88	0.02	2.99	3.00	2.95	2.98	0.03	3.01	3.04	3.05	3.03	0.02	0.25	0.15
2.00 log10 IU/mL	2.00	1.88	1.88	1.92	0.07	2.00	1.99	1.85	1.95	0.08	2.04	2.12	2.05	2.07	0.04	0.24	0.15

- NeuMoDx CMV Assay is an in vitro diagnostic test for quantitative detection of Human Cytomegalovirus (CMV) DNA in human plasma.
- Three lots of CMV Test Strips were stored at 50°C up to 61 days, equivalent to 13 months at 23°C.
- The activity of the strips were accessed by testing the aged strips on the NeuMoDx System using human plasma spiked with CMV at concentrations of 5, 3 and 2 log10 IU/mL (6 replicates per level) per lot for each storage condition.
- There is minimum drift, ≤ 0.25%, in quantification of CMV after storage for 61 days at 50°C (equivalent to 13 month when stored at 23°C) with 100% detection rate at all the levels.
- Three lots of test strips performed reproducibly with SD < 0.07 Log10 IU/mL across all the testing points and concentrations.
- Representative PCR amplification curves from one of the three lots of HCV Test Strips are shown above.

NEUMODX HCV QUANT ASSAY TEST STRIP: ACCELERATED STUDY





HCV Expected Conc, [Log10 IU/mL]	D0		D44 (Eq 5 months)		D70 (Eq 8 months)		D96 (Eq 11 months)		D149 (Eq 17 months)		Δ fr D0	% Drift
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD		
5.00	5.16	0.03	4.82	0.07	4.89	0.02	4.90	0.15	5.00	0.17	-0.16	0.07%
3.00	3.11	0.05	2.76	0.11	2.81	0.02	2.86	0.09	2.94	0.08	-0.17	0.07%
1.88	1.86	0.10	1.61	0.06	1.65	0.07	1.69	0.12	1.76	0.17	-0.11	0.04%

- NeuMoDx HCV Assay is a in vitro diagnostic for quantitative detection of Human Hepatitis C Virus (HCV) RNA in human plasma and serum.
- Three lots of HCV Test Strips were stored at 40°C up to 149 days, equivalent to 17 months at 23°C.
- The activity of the strips was evaluated by testing the aged strips on the NeuMoDx System using human plasma spiked with HCV at concentrations of 5, 3 and 1.88 log10 IU/mL (6 replicates per level) per lot for each storage condition.
- After storage up to 149 days at 40°C, the test strips quantified HCV targets with minimum bias to the expected concentrations. The difference between D149 and D0 is < 0.17 Log10 IU/mL and almost no drift, ≤ 0.07%, was observed when analyzed with linear regression.
- Three lots of test strips performed reproducibly with SD < 0.17 Log10 IU/mL across all the testing points and concentrations.
- Representative PCR amplification curves from one of the three lots of HCV Test Strips are shown above.

CONCLUSION

NeuDry reagents demonstrated excellent nucleic acid isolation from several clinical matrices as well as efficient and robust PCR/RT-PCR amplification using the eluted DNA/RNA directly. In addition, the NeuDry reagents have demonstrated a long shelf life of 25 months with an in-use life of 63 days.

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.