ANALYTICAL PERFORMANCE OF THE NEUMODX CTNG TEST ON THE NEUMODX MOLECULAR SYSTEMS FOR DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* FROM URINE AND SWAB SPECIMENS

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INTRODUCTION

Laboratories around the world rely on automated nucleic acid testing systems (NAATs) for screening and diagnosis of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections in symptomatic/asymptomatic patients from urine and swab matrices. The NeuMoDx CTNG Test is CEIVD certified for use with urine specimens on the high-throughput floor model NeuMoDx 288 Molecular System (N288). The goal of this study was to demonstrate performance of the NeuMoDx CTNG Test on the medium throughput, bench-top NeuMoDx 96 System as well as across additional specimen types such as vaginal and endocervical swabs. Results of verification of these studies in both urine and swab matrices on both NeuMoDx Platforms are presented here.

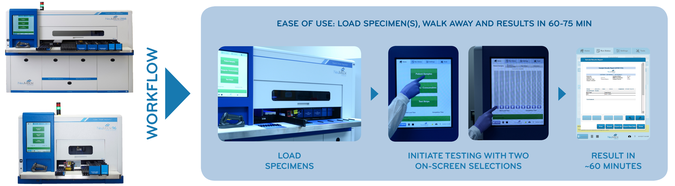
METHODS

Analytical studies were performed in urine and both swab matrices across the NeuMoDx 288 and NeuMoDx 96 systems to characterize various analytical parameters such as – sensitivity, cross-reactivity, inclusivity, impact of interfering substances, cross-contamination, specimen stability, and turnaround time (TAT).

RESULTS

The NeuMoDx CTNG Test demonstrated a Limit of Detection of 9 EB/mL for CT and 5 cells/mL for NG in urine, and 20 EB/mL for CT and 5 cells/mL for NG in swabs with excellent inclusivity across the 15 CT serovars and ~10 NG isolates. The sensitivity of each target (CT or NG) was maintained in the presence of high target concentration (>1E6 cells/mL or EB/mL) of the other target (NG or CT) demonstrating the robustness of the assay. 100% specificity was observed across ~113 phylogenetically similar or co-habiting pathogens; and the test demonstrated no adverse effect in the presence of ~150 endogenous/ exogenous interfering agents or pathogens. The NeuMoDx CTNG Test demonstrated excellent precision across 3 instruments over 12 days test days, as well as excellent reproducibility across multiple reagent lots. Time to first results was only 60 minutes enabling delivery of rapid results for emergency samples and the specimens were stable for at least 24 hours on board the worktable of the instrument enabling walkaway operation.

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 DNA tests in an 8 hour shift for the N288, ~150 DNA tests in an 8 hour shift for the N96
- **Fast Time to First Results:** ~60 min
- **Continuous Loading:** 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.**

Analytical Sensitivity (LoD)

The Limit of Detection of the NeuMoDx CTNG Test in the urine matrix was determined with pooled negative clinical urine spiked with AcroMetrix™ CT or NG at six different levels including negative samples.

The Limit of Detection of the NeuMoDx CTNG Test in swab matrices was determined in a hit-rate style with pooled negative clinical vaginal or endocervical swabs spiked with AcroMetrixTM CT or NG at three different levels including negative samples.

The limit of detection in urine was determined to be 6 EB/mL for CT and 5 cells/mL for NG (after interference studies, as well). The limit of detection in swabs was determined to be 20 EB/mL for CT and 5 cells/mL for NG.

NeuMoDx CTNG Test Limit of Detection - CT				
Target Conc. (EB/mL)	N	# Positive	% Positive	LoD (Detection)
N288 Results				
32	60	60	100%	4.5 EB/mL (95% CI 3.8-5.2 EB/mL)
16	60	60	100%	
8	60	60	100%	
4	59	54	92%	
2	60	38	63%	
0	60	0	0%	
N96 Results				
32	24	24	100%	7 EB/mL* (95% CI 4.5- 26 EB/mL)
16	24	24	100%	
8	24	23	96%	
4	23	19	83%	
2	24	18	75%	
0	20	0	0%	

NeuMoDx CTNG Test Limit of Detection - NG				
Target Conc. (cells/mL)	N	# Positive	% Positive	LoD (Probit)
N288 Results				
10	58	58	100%	0.25 cells/mL (95% CI (0.25, 0.25) cells/mL)
5	60	60	100%	
1	60	60	100%	
0.3	59	58	98%	
0.1	59	37	64%	
0	59	0	0%	
N96 Results				
10	24	24	100%	0.3 cells/mL (95% CI (0.28, 0.28) cells/mL)
5	24	24	100%	
1	24	24	100%	
0.3	24	23	96%	
0.1	24	11	46%	
0	22	0	0%	

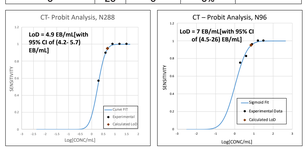


Table 1. Limit of Detection of the NeuMoDx CTNG Test in the urine matrix for the CT target. The overall LoD of the CT target in urine was determined to be 6 EB/mL.

*N96 instrument LoD was deemed acceptable due to smaller sample size

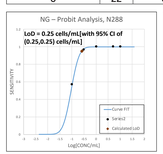
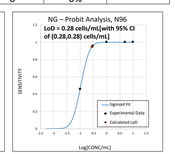


Table 3. Limit of Detection of the NeuMoDx CTNG Test in the swab matrix for the CT target. The overall LoD of the CT target in swab was determined to be 20 EB/mL.



NeuMoDx CTNG Test Limit of Detection – CT (N288 & N96)				
Target Conc. (EB/mL)	N	# Positive	% Positive	LoD (Probit)
Vaginal Swab Results				
30	48	48	100%	20 EB/mL
20	48	48	100%	
0	48	0	0%	
Endocervical Swab Results				
30	48	48	100%	20 EB/mL
20	48	48	100%	
0	48	0	0%	

Table 2. Limit of Detection of the NeuMoDx CTNG Test in the swab matrix for the CT target. The overall LoD of the CT target in swab was determined to be 20 EB/mL.

NeuMoDx CTNG Test Limit of Detection – NG (N288 & N96)				
Target Conc. (cells/mL)	N	# Positive	% Positive	LoD (Detection)
Vaginal Swab Results				
9	48	48	100%	5 cells/mL
5	48	47	98%	
0	48	0	0%	
Endocervical Swab Results				
9	48	48	100%	5 cells/mL
5	48	48	100%	
0	48	0	0%	

Table 4. Limit of Detection of the NeuMoDx CTNG Test in the swab matrix for the NG target. The overall LoD of the NG target in swab was determined to be 5 cells/mL.

CT Serovar	NG Clinical Isolate (ATCC #)
A	49981
B	31426
Ba	31407
C	27633
LGV I	9793
LGV II	43070
LGV III	51109
E	35542
F	35541
G	49498
H	49926
I	
J	
K	

Table 5. Limit of Detection of the NeuMoDx CTNG Test in the swab matrix for the NG target. The overall LoD of the NG target in swab was determined to be 5 cells/mL.

Analytical Specificity & Interference

Analytical Specificity & Cross-Reactivity

A total of 113 culture isolates or DNA from organisms potentially cohabiting or phylogenetically similar to either CT or NG were evaluated for possible cross-reactivity with the NeuMoDx CTNG Test. Most of the organisms were spiked into CTNG negative urine at ~1E6 CFU/mL, except in the case of a few organisms where high copies of DNA (10 ng/mL) were spiked into CTNG negative urine. The list of organisms tested is shown in Table 6. In addition, it was demonstrated that the detection of CT and NG targets, even at low levels of target (~3X LoD), was not affected by the presence of any of these organisms.

Bacteria	
Anaerobic	12
Candida	4
Chlamydia	1
Enterococcus	3
Lactobacillus	4
Mycoplasma	2
Neisseria	15
Pseudomonas	2
Salmonella	2
Staphylococcus	3
Streptococcus	8
Trichomonas	1
Ureaplasma	1
And other bacteria	51
Virus	4

Neisseria meningitidis Serogroup A
Neisseria meningitidis Serogroup B
Neisseria meningitidis Serogroup D
Neisseria meningitidis Serogroup Y
Neisseria meningitidis Serogroup W135
Neisseria cinerea
Neisseria elongate
Neisseria flavescens
Neisseria lactamica
Neisseria mucosa
Neisseria sicca
Neisseria subflava
Neisseria perflava
Neisseria polysaccharea

Table 6. List of organisms used in the analytical specificity and cross-reactivity studies for the NeuMoDx CTNG Test. All organisms tested produced a negative result in absence of either CT or NG target. Additionally, the presence of these organisms at low levels did not affect the detection of CT or NG.

Interfering Substances

The performance of the NeuMoDx CTNG Test in the presence of endogenous/ exogenous substances was evaluated by testing for efficacy of detection of low (3x LoD) and moderate (10x LoD) level CT & NG targets. This was done in both negative clinical urine and swab matrix, with endocervical and vaginal swabs being tested separately. Across all matrices, a total of 10 endogenous conditions and 6 exogenous substances were evaluated. Spiked samples were monitored for positivity and threshold inhibition. No interference was seen from the substance concentrations listed in Table 7.

NeuMoDx CTNG Test - Endogenous/Exogenous		
Urine	3x LoD	10x LoD
Blood, 7%	100%	100%
Bilirubin, >1 mg/dL*	100%	100%
Glucose, 1000 mg/dL	100%	100%
Acidic Urine, pH 4	100%	100%
Alkaline Urine, pH 9	100%	100%
Protein (Albumin), 50 mg/mL	100%	100%
PBMCs, 1x10^6 cells/mL	100%	100%
Talcum Powder, 1%	100%	100%
Swab	3x LoD	10x LoD
Blood, 10%	100%	100%
Mucin, 12.9 mg/mL**	100%	100%
PBMCs, 1x10^5	100%	100%
Monistat 1, 21.2 mg/mL	100%	100%
KY Jelly Lubricant, 42.2 mg/mL	100%	100%
Yeast Gard Douche, 31.1 mg/mL	100%	100%
Progesterone, 5.9 mg/mL	100%	100%
Vagisil, 7.1 mg/mL	100%	100%
Seminal Fluid, 12.9 mg/mL	100%	100%

Table 7. The NeuMoDx CTNG Test was performed with CTNG positive urine and swab matrix prepared by spiking negative matrix with CTNG targets at 3x LoD and 10x LoD in the presence of the listed interfering substances. Urine, n=3, Swab n=4.

*Disease state clinical specimen
**Dosed from a 0.8% stock

Cross-Contamination

The vulnerability of the NeuMoDx CTNG Test in conditions inclined to high levels of contamination was initially evaluated for cross contamination by performing a full run with alternating very high CTNG-positive urine and CTNG-negative urine in a "checkerboard" pattern. The possibility for carryover contamination was evaluated by running a full run of very high positive sample followed by a full run of negative samples. Both of these conditions were tested on two different NeuMoDx 288 Molecular Systems. For all valid results, no amplification was seen in any negative sample and all positive samples were correctly called positive for both targets.

NeuMoDx CTNG Test in Urine-Contamination							
System	Level	#POS Results	#NEG Results	#IND Results	#UNR Results	Total #Valid Results	Total # Results
CROSS CONTAMINATION							
N000001	High Positive	24	0	0	0	24	24
	Negative	0	23	0	0	23	23
N000004	High Positive	24	0	0	0	24	24
	Negative	0	23	0	1	23	24
TOTAL	High Positive	48	0	0	0	48	48
	Negative	0	46	0	1	46	47
CARRYOVER CONTAMINATION							
N000001	High Positive	46	0	1	0	46	47
	Negative	0	47	0	0	47	47
N000004	High Positive	48	0	0	0	48	48
	Negative	0	46	2	0	46	48
TOTAL	High Positive	94	0	1	0	94	95
	Negative	0	93	2	0	93	95

Table 8. Contamination data for both cross contamination and carryover contamination. All valid results show complete absence of contamination in either condition.

Specimen Stability

The NeuMoDx CTNG Test in Swab was evaluated for successful stability of specimens stored in normal refrigerator conditions with CTNG-negative clinical vaginal and endocervical swabs spiked with purified *Chlamydia trachomatis* (CT) elementary bodies (EB) and cultured *Neisseria gonorrhoeae* (NG) cells and stored between 2-8°C. At 30 days, 100% concordance with time 0 results was seen. Studies to assess specimen stability beyond 30 days are currently ongoing.

Specimen Stability of the NeuMoDx CTNG Test-Vaginal Swab						
		Confirmed CT Positive Samples		Confirmed NG Positive Samples		Confirmed Negative Samples
Timepoint		#Positive	#Negative	#Positive	#Negative	#Positive	#Negative
1	Time 0	32/32	0/32	32/32	0/32	0/16	16/16
2	4 Days	32/32	0/32	32/32	0/32	0/15	15/15
3	7 Days	32/32	0/32	30/30	0/30	0/16	16/16
4	14 Days	32/32	0/32	32/32	0/32	0/16	16/16
5	30 Days	32/32	0/32	32/32	0/32	0/16	16/16
% Concordance		100%		100%		100%	

Table 9. The stability of the NeuMoDx CTNG Test in Swab was evaluated at increasing timepoints to assess test accuracy after sample storage at 2-8°C. Invalid samples were rerun until a valid result was obtained, and all results were processed with the most up-to-date assay definition parameters.

Specimen Stability of the NeuMoDx CTNG Test-Endocervical Swab						
		Confirmed CT Positive Samples		Confirmed NG Positive Samples		Confirmed Negative Samples
Timepoint		#Positive	#Negative	#Positive	#Negative	#Positive	#Negative
1	Time 0	32/32	0/32	32/32	0/32	0/16	16/16
2	4 Days	32/32	0/32	32/32	0/32	0/16	16/16
3	7 Days	32/32	0/32	32/32	0/32	0/16	16/16
4	14 Days	32/32	0/32	32/32	0/32	0/16	16/16
5	30 Days	32/32	0/32	32/32	0/32	0/16	16/16
% Concordance		100%		100%		100%	

Table 10. The stability of the NeuMoDx CTNG Test in Swab was evaluated at increasing timepoints to assess test accuracy after sample storage at 2-8°C. Invalid samples were rerun until a valid result was obtained, and all results were processed with the most up-to-date assay definition parameters.

Specimen Stability of the NeuMoDx CTNG Test-Urine						
		Confirmed CT Positive Samples		Confirmed NG Positive Samples		Confirmed Negative Samples
Timepoint		#Positive	#Negative	#Positive	#Negative	#Positive	#Negative
1	Time 0	10/10	10/10	10/10	10/10	0/10	10/10
2	4 Days	10/10	10/10	10/10	10/10	0/10	10/10
3	7 Days	10/10	10/10	10/10	10/10	0/10	10/10
% Concordance		100%		100%		100%	

Table 11. The stability of the NeuMoDx CTNG Test in Urine was evaluated at increasing timepoints to assess test accuracy after sample storage at 2-8°C.

Turnaround Time

The NeuMoDx CTNG Test on the NeuMoDx Molecular System was assessed for efficiency in turnaround time, which is defined as the time in minutes for samples to return a 'Completed' result after loading on the System for sample processing. Eight samples of CTNG-dosed urine were run on two different NeuMoDx Molecular Systems. Elapsed time was measured by checking the system reported start and end times, as well as a manual measurement with a handheld timer. All samples were completed in under an hour on both machines.

Turnaround Time of the NeuMoDx CTNG Test						
Machine	Method	Start Time	End Time	Elapsed Time	Overall Results	Replicates
N000002	System Log	10:08 am	11:07 am	59 min.	Positive	n=8
	Timer	0:00	0:58:40	~59 min.		
N000005	System Log	10:09 am	11:07 am	58 min.	Positive	n=8
	Timer	0:00	0:58:42	~59 min.		

Table 12. Time elapsed was calculated from exported results on both machines. A manual measurement with a laboratory timer was also tracked. Runs on both machines completely processed 8 samples in less than 60 minutes.

Method Comparison Studies

The comparative performance of the NeuMoDx CTNG Test to an outside laboratory's FDA-cleared CTNG molecular diagnostic test was assessed for accuracy in both urine and swab matrices. The outside laboratory's diagnostic test was performed on individual patient specimens, and the residuals of these samples were then sent to NeuMoDx Molecular. Residual samples were analyzed by the NeuMoDx CTNG Test on two different NeuMoDx Molecular Systems. Results from NeuMoDx testing were compared to outside laboratory results to assess the NeuMoDx CTNG Test's sensitivity and specificity.

In urine, the overall sensitivity for the Chlamydia target was found to be 96.7% with a specificity of 99.7%. The Gonorrhea target in urine was found to have a sensitivity of 98.1% and a specificity of 99.7%.

Swab samples were analyzed separately from urine, as well as being separated by type (endocervical vs. vaginal swabs). The overall sensitivity for both types of swab at the Chlamydia target was 100%, with a specificity of 99.6%. The Gonorrhea target saw a sensitivity of 100% and a specificity of 98.7% for all swab types combined. The accompanying tables show a further breakdown of sensitivity and specificity by swab type.

Method Comparison of the NeuMoDx CTNG Test-Urine				
Chlamydia		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	87	3	90
	NEG	1	297	298
	TOTAL	88	300	388
Sensitivity = 96.7% 95% CI (90.7% - 98.9%)				
Specificity = 99.7% 95% CI (98.1% - 99.9%)				

Method Comparison of the NeuMoDx CTNG Test-Urine				
Gonorrhea		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	51	1	52
	NEG	1	335	336
	TOTAL	52	336	338
Sensitivity = 98.1% 95% CI (88.4% - 99.9%)				
Specificity = 99.7% 95% CI (98.1% - 100%)				

Table 13. Truth tables for the results of the NeuMoDx CTNG Test in comparison to FDA approved tests from reference labs as CT results and NG results.

Method Comparison of the NeuMoDx CTNG Test-Swab, Overall				
Chlamydia		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	62*	0	62
	NEG	1	263	264
	TOTAL	63	263	326
Sensitivity = 100% 95% CI (92.7-100%)				
Specificity = 99.6% 95% CI (97.6-100%)				

Method Comparison of the NeuMoDx CTNG Test-Swab, Overall				
Gonorrhea		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	103*	0	103
	NEG	3	220	220
	TOTAL	106	220	326
Sensitivity = 100% 95% CI (95.5-100%)				
Specificity = 98.7% 95% CI (95.8-99.7%)				

*24 of these specimens were a part of a contrived panel.

*94 of these specimens were a part of a contrived panel.

Table 14. Truth tables for the results of the NeuMoDx CTNG Test in comparison to FDA approved tests from reference labs for CT and NG targets (overall results for both swab types combined).

Method Comparison of the NeuMoDx CTNG Test-Endocervical Swabs				
Chlamydia		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	37*	0	37
	NEG	1	130	131
	TOTAL	38	130	168
Sensitivity = 100% 95% CI (88.3-100%)				
Specificity = 99.2% 95% CI (95.2-100%)				

Method Comparison of the NeuMoDx CTNG Test-Endocervical Swabs				
Gonorrhea		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	52*	0	52
	NEG	1	115	116
	TOTAL	53	115	168
Sensitivity = 100% 95% CI (91.4-100%)				
Specificity = 99.1% 95% CI (94.6-100%)				

*12 of these specimens were a part of a contrived panel.

*46 of these specimens were a part of a contrived panel.

Table 15. Truth tables for the results of the NeuMoDx CTNG Test in comparison to FDA approved tests from reference labs for CT and NG targets (results for endocervical swabs only).

Method Comparison of the NeuMoDx CTNG Test-Vaginal Swabs				
Chlamydia		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	25	0	25
	NEG	0	133	133
	TOTAL	25	0	158
Sensitivity = 100% 95% CI (83.4-100%)				
Specificity = 100% 95% CI (96.5-100%)				

Method Comparison of the NeuMoDx CTNG Test-Vaginal Swabs				
Gonorrhea		NeuMoDxTest		
		POS	NEG	TOTAL
Reference Test	POS	51*	0	51
	NEG	2	105	107
	TOTAL	53	105	158
Sensitivity = 100% 95% CI (93.3-100%)				
Specificity = 98.1% 95% CI (92.8-99.7%)				

*12 of these specimens were a part of a contrived panel.

*48 of these specimens were a part of a contrived panel.

Table 16. Truth tables for the results of the NeuMoDx CTNG Test in comparison to FDA approved tests from reference labs for CT and NG targets (results for vaginal swabs only).

CONCLUSIONS & ACKNOWLEDGMENTS

The NeuMoDx CTNG Test provides an extremely easy to use, reliable, low-cost, and rapid method for detection and differentiation of CT and NG directly from urine and swab specimens. *For Research Use Only. Not for use in diagnostic procedures.* | The authors gratefully acknowledge the help and support provided by all members of the NeuMoDx team. We would also like to thank our collaborators: Beaumont Health, Indiana University School of Medicine, Tampa General Hospital and Laboratory Alliance for their invaluable assistance in providing access to clinical specimens for testing and evaluation.