PERFORMANCE OF A RAPID GROUP A STREP XCR ASSAY ON THE FULLY AUTOMATED NEUMODX MOLECULAR SYSTEM

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BACKGROUND

Streptococcus pyogenes, also known as Group A streptococcus (GAS), is responsible for a range of diseases including strep throat, impetigo, cellulitis and more severe illnesses such as necrotizing fasciitis and toxic shock syndrome. It is mainly spread via respiratory droplets in the air or through close contact. Diagnosis has traditionally been performed through rapid antigen detection test or throat culture. Both current approaches suffer from significant drawbacks. Rapid antigen tests suffer from poor specificity and the culture identification takes at least 24-48 hours. The NeuMoDx GAS Test is a "sample to result" type nucleic acid test incorporating XCR - a novel ultra-fast amplification technology – implemented on the fully automated NeuMoDx Molecular System. All the reagents and consumables used are stable at ambient conditions, do not require any refrigeration, and come in ready-to-use configurations requiring no user mediated steps.

MATERIALS/METHODS

Performance of the GAS Test was characterized from throat swabs in both liquid Amies as well as a "dry" Amies transport system. Studies were performed to characterize the analytical sensitivity of both the M1 and M3 GAS strains, analytical specificity across non-target organisms, robustness in the presence of interfering substances, and clinical sensitivity and specificity by performing a pilot method comparison study against a commercially available comparator test.

RESULTS

The Limit of Detection of the NeuMoDx GAS Test was established to be 70 CFU/mL in both the M1 and M3 Strains demonstrating excellent sensitivity. No cross-reactivity was observed against any of the 21 phylogenetically similar or co-habiting non-target organisms in throat swab specimens tested, showing 100% analytical specificity. No interference was demonstrated in the presence of 26 relevant endogenous and exogenous substances and pathogens. Turnaround time for the complete test was only ~40 min with the amplification and detection process only taking ~13 minutes. Finally, excellent sensitivity (97.9%) and specificity (99.1%) was demonstrated in a pilot method comparison study using 157 de-identified residual clinical throat swab specimens as compared to a reference test.

Analytical Specificity (Cross-Reactivity) & Interference

A total of 21 non-target organisms potentially cohabiting or phylogenetically similar to GAS were evaluated for possible cross-reactivity with the NeuMoDx GAS Test. No cross-reactivity with these 21 organisms was seen.

Additionally, no interference was seen with the NeuMoDx GAS Test in the presence of 26 relevant endogenous/ exogenous substances or organisms.

The bacteria were added to >1E6 CFU/ mL and the viruses at 1E6-1E7 IU/mL. The list of these organisms is shown in Table 3.

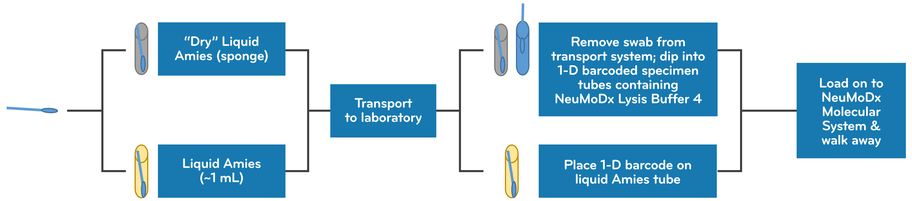
Table 3. List of organisms used in the analytical specificity and cross-reactivity studies for the NeuMoDx GAS Test. No cross-reactivity or interference was observed with any of the organisms tested.

Pathogen	Catalog/Reference #
Streptococcus Mitis	BEI: HM 262
Streptococcus Salivanus	ATCC: BAA 491
Streptococcus Sanguinis	ATCC: 10556
Streptococcus Mutans	ATCC: 25715
Streptococcus Pneumoniea	ATCC: 49619
Streptococcus intermedius	BEI: HM 368
Streptococcus bovis	BEI: HM 272
Streptococcus anginosus	BEI: HM 282
Streptococcus parasamguinis	BEI: HM 808
Streptococcus uberis	ATCC: 700407
Streptococcus canis	ATCC: 43496
Streptococcus oralis	ATCC: 9811
Streptococcus dysgalactiae	ATCC: 12394
Staphylococcus Aureus	ATCC: 27660
Staphylococcus Epidermidis	ATCC: 700576
Staphylococcus saprophyticus	ATCC: 15305
Group B Streptococcus	ATCC: BAA 611
Staphylococcus sp.	ATCC: 155
RSV RNA	ATCC: 1540D
Chlamydophilia pneumoniae (AR-39)	53592D
Influenza Virus (H3N2)	VR 544

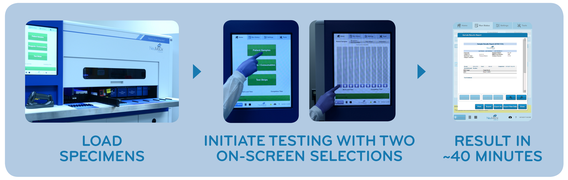
Interfering Substances Test				
Interfering Moiety	Concentration	Result	Gas Ct Average	SPC Ct Average
Human Blood	5%	POSITIVE	33.50	34.55
Saliva	50 uL/swab	POSITIVE	35.78	35.43
Mucin	2.5 mg/mL	POSITIVE	35.22	35.36
Toothpaste	5% (v/v)	POSITIVE	37.15	36.64
Mouthwash	5% (v/v)	POSITIVE	37.15	36.64
Positive Control Result				
Positive Control	N/A	POSITIVE	33.84	35.68

Table 4. GAS Interfering Substances – Endogenous/Exogenous Substances. The possible interference of relevant endogenous and exogenous substances on NeuMoDx GAS Test was evaluated with five (5) substances listed in Table 4. The NeuMoDx GAS Test did not demonstrate any interference in the presence of any of the substances tested.

NeuMoDx GAS Test Work Flow



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
- **Large Walk-Away Window:** Up to 288 samples for the N288, 96 samples for the N96
- **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 of Specimens:** 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

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)

Limit of Detection

The preliminary Limit of Detection of the NeuMoDx GAS Test was determined with pooled negative clinical specimens spiked with cultured GAS M1 strain at six different levels including negative samples.

The sensitivity was further confirmed by a hit-rate study with pooled negative clinical specimens spiked with 70 CFU/mL of GAS. The established limit of detection was also confirmed using the GAS M3 strain.

The limit of detection was determined to be 70 CFU/ mL for GAS M1 and M3 strains.

Preliminary NeuMoDx GAS Test Limit of Detection (GAS M1)				
Matrix	GAS Conc.(CFU/mL)	N	# Positive	% Positive
	N288 Results			
Pooled Clinical Negative Throat Swabs	500	3	3	100%
	200	3	3	100%
	100	3	3	100%
	70	3	3	100%
	50	3	3	100%
	0	3	0	0%
Liq Amies (controls)	70	3	3	100%
	0	3	0	0%

Table 1. Limit of Detection of the NeuMoDx GAS Test in the throat swab matrix for the GAS M1 strain. The overall LoD of the GAS M1 target was determined to be 70 CFU/mL.

Confirmation, Hit-Rate for GAS M1 Strain					Confirmation, Hit-Rate for GAS M1 Strain				
Matrix	GAS Conc (CFU/mL)	N	# Positive	% Positive	Matrix	GAS Conc (CFU/mL)	N	# Positive	% Positive
Pooled Clinical Negative Throat Swabs	70	65	65	100%	Pooled Clinical Negative Throat Swabs	70	41	50	97.56%

Table 2. Limit of Detection of the NeuMoDx GAS Test for the GAS M1 and M3 strains was confirmed in a hit-rate study to be 70 CFU/mL.

Method Comparison

The clinical sensitivity and specificity of the NeuMoDx GAS Test was assessed against the comparator test, Lyra Direct Strep by NAA (Quidel) by testing 157 clinical throat swabs collected in liquid Amies from GAS-tested patients.

Testing was performed internally at NeuMoDx through a single-blinded study using de-identified residual clinical samples obtained from reference laboratories. Among the 107 "True" negatives, 9 were Group C/G Streptococcus positive by the comparator's test, indicating no cross-reactivity with group C/G for the NeuMoDx GAS Test.

The NeuMoDx GAS Test demonstrated excellent clinical sensitivity and specificity in this pilot method comparison study.

		Quidel Lyra™ Direct Strep		
		Positive	Negative	Total
NeuMoDx GAS Test	Positive	47	1	48
	Negative	1	106	107
	Total	48	107	155
Clinical Sensitivity		97.92% CI 95% (89.10 – 99.63)		
Clinical Specificity		99.07% CI 95% (94.90 – 99.84)		

Table 5. NeuMoDx GAS Test Method Correlation. The NeuMoDx GAS Test demonstrated excellent clinical sensitivity and specificity with the comparator GAS test results.

CONCLUSIONS

The NeuMoDx GAS Test is a rapid, easy to use test for direct detection of Group A Strep from throat swabs, providing results in <40 minutes.
For Research Use Only. Not for use in diagnostic procedures.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help and support provided by all members of the NeuMoDx team. We would also like to thank our collaborators: XCR Diagnostics for use of their XCR chemistry and Beaumont Health for their invaluable assistance in providing access to clinical specimens for testing and evaluation.