

PERFORMANCE OF A RAPID MULTIPLEX STREP ASSAY ON THE FULLY AUTOMATED NEUMODX MOLECULAR SYSTEMS

Brad Keusch, Andrew Narwold, Bryson Green*, Lijie Gong, Mark Olson, Maureen Carey, Aaron Ripley, Michelle Mastronardi, Daniel Kolk*, Betty Wu, and Sundu Brahmasandra, NeuMoDx Molecular, Ann Arbor MI, *XCR Diagnostics, Salt Lake City, UT

BACKGROUND Group A β-hemolytic streptococci (GAS) are the most frequently isolated pathogens in acute bacterial pharyngitis. In addition, Group C and G Streptococcus (GCS/GGS) have been increasingly recognized as potential causes of non-group A Streptococcal pharyngitis in children. Diagnosis has traditionally been performed through rapid antigen detection tests or culture. Both approaches suffer from significant drawbacks. Rapid antigen tests for GAS suffer from poor specificity and culture identification takes at least 24–48 hours. The NeuMoDx Strep A/C/G Vantage Assay is a multiplex, "sample to result" nucleic acid test incorporating XCR—a novel ultra-fast amplification technology—implemented on the fully automated, and consumables used are stable at ambient conditions, do not require any refrigeration, and come in ready-to-use configurations requiring

high-throughput NeuMoDx Molecular Systems. It detects and differentiates DNA of GAS and GCS/GGS simultaneously. All the reagents no user-mediated steps.

METHODS Performance of the Strep Assay was characterized from clinical throat swabs in Liquid Amies and in neat Liquid Amies. Studies were performed to characterize the analytical sensitivity of both the M1 and M3 GAS strains, as well as GCS and GGS. Additionally, analytical specificity across non-target organisms, robustness in the presence of interfering substances, and clinical sensitivity and specificity (by performing a method comparison study against commercially available comparator tests) were determined for the NeuMoDx Strep Assay.

RESULTS The Limit of Detection of the NeuMoDx Strep A/C/G Vantage Assay was established to be 50 and 100 CFU/mL for the GAS M1 and M3 strains respectively, 2.5E3 CFU/mL for GCS and 1E4 CFU/mL for GGS. The assay displayed 100% analytical specificity with no cross-reactivity observed against any of the 45 phylogenetically similar or co-habiting non-target organisms. No interference was demonstrated in the presence of relevant endogenous and exogenous substances and pathogens. Turnaround time for the complete test was only ~50 min with the amplification and detection process only taking ~13 minutes. Finally, excellent sensitivity (GAS: 100%, GCS/GGS: 95.9%) and specificity (100%) were demonstrated in a method comparison study using 230 clinical residual specimens as compared to a reference test.

Limit of Detection

GAS Strain	n	CFU/mL	% Detected
Streptococcus pyogenes, M1*	40	50	100%
Streptococcus pyogenes, M2	20	300	100%
Streptococcus pyogenes, M3	5	100	100%
Streptococcus pyogenes, M82	5	100	100%
Streptococcus pyogenes, M4	5	100	100%
Streptococcus pyogenes, M18	20	100	95%
Streptococcus pyogenes, M28	20	300	95%
Streptococcus pyogenes, M73	20	500	100%
Streptococcus pyogenes, M78	20	500	100%
Streptococcus pyogenes, M77	19	500	100%
Streptococcus pyogenes, M12	20	500	100%
Streptococcus pyogenes, M75	20	1500	100%
Streptococcus pyogenes, M49	20	2500	95%

GCS Strain	n	CFU/mL	% Detected
Streptococcus dysgalactiae subsp. Equisimilis (ATCC 35666)*	40	2500	100%
Streptococcus dysgalactiae subsp. Equisimilis, C74	5	5000	100%
Streptococcus dysgalactiae subsp. Equisimilis strain 13-166	5	5000	100%
<i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis strain</i> LRA 06 11 76 [NCTC 8543, SS660]	5	5000	100%
Streptococcus dysgalactiae subsp. Equisimilis strain 1180	5	5000	100%
Streptococcus dysgalactiae subsp. Equisimilis, C46	5	5000	100%
<i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis strain</i> UCM 74/02P	5	5000	100%
Streptococcus dysgalactiae subsp. Equisimilis strain SVA XVI 172	5	5000	100%
Streptococcus dysgalactiae subsp. Equisimilis strain STR 655	20	15000	100%
GGS Strain	n	CFU/mL	% Detected
GGS Strain <i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis (</i> ATCC 12394)*	n 40	CFU/mL 10000	% Detected 100%
GGS Strain Streptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)* Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129	n 40 5	CFU/mL 10000 10000	% Detected 100% 100%
GGS Strain <i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis</i> (ATCC 12394)* <i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis</i> , strain NIH 1129 <i>Streptococcus dysgalactiae</i> subsp. <i>Equisimilis</i> , G16	n 40 5 5	CFU/mL 10000 10000 10000	% Detected 100% 100% 100%
GGS StrainStreptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)*Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129Streptococcus dysgalactiae subsp. Equisimilis, G16Streptococcus dysgalactiae subsp. Equisimilis, G16	n 40 5 5 5 5	CFU/mL 10000 10000 10000	% Detected 100% 100% 100%
GGS StrainStreptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)*Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129Streptococcus dysgalactiae subsp. Equisimilis, G16Streptococcus dysgalactiae subsp. Equisimilis - CCUG 15679Streptococcus dysgalactiae subsp. Equisimilis, G47	n 40 5 5 5 5 5	CFU/mL 10000 10000 10000 10000	% Detected 100% 100% 100% 100%
GGS StrainStreptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)*Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129Streptococcus dysgalactiae subsp. Equisimilis, G16Streptococcus dysgalactiae subsp. Equisimilis - CCUG 15679Streptococcus dysgalactiae subsp. Equisimilis, G47Streptococcus dysgalactiae subsp. Equisimilis, G47	n 40 5 5 5 5 5 5 5	CFU/mL 10000 10000 10000 10000 10000	% Detected 100% 100% 100% 100% 100% 100%
GGS StrainStreptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)*Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129Streptococcus dysgalactiae subsp. Equisimilis, G16Streptococcus dysgalactiae subsp. Equisimilis - CCUG 15679Streptococcus dysgalactiae subsp. Equisimilis, G47Streptococcus dysgalactiae subsp. Equisimilis, G47Streptococcus dysgalactiae subsp. Equisimilis, CCUG 27483Streptococcus dysgalactiae subsp. Equisimilis, CCUG 33802	n 40 5 5 5 5 5 5 5 5 5	CFU/mL 10000 10000 10000 10000 10000 10000	% Detected 100% 100% 100% 100% 100% 100% 100%
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GGS StrainStreptococcus dysgalactiae subsp. Equisimilis (ATCC 12394)*Streptococcus dysgalactiae subsp. Equisimilis, strain NIH 1129Streptococcus dysgalactiae subsp. Equisimilis, G16Streptococcus dysgalactiae subsp. Equisimilis - CCUG 15679Streptococcus dysgalactiae subsp. Equisimilis, G47Streptococcus dysgalactiae subsp. Equisimilis - CCUG 27483Streptococcus dysgalactiae subsp. Equisimilis - CCUG 33802Streptococcus dysgalactiae subsp. Equisimilis, CCUG 33802Streptococcus dysgalactiae subsp. Equisimilis - CCUG 502Streptococcus dysgalactiae subsp. Equisimilis - CCUG 15680	n 40 5 5 5 5 5 5 5 5 5 5 5 5 5 5	CFU/mL 10000 10000 10000 10000 10000 10000 10000 20000	% Detected 100% 100% 100% 100% 100% 100% 100% 100% 100% 100%

Model Strain - Limit of Detec

Streptococcus pyogenes, M1 (ATCC 700294)

Streptococcus dysgalactiae subs Equisimilis (ATCC 35666)

Streptococcus dysgalactiae subs Equisimilis (ATCC 12394)

The Limit of Detection (LoD) of the NeuMoDx Strep A/C/G Vantage Assay in clinical throat swab matrix was evaluated by testing 40 replicates of each Strep model strain (GAS, GCS, and GGS) at 50 CFU/mL, 2500 CFU/ mL, and 10,000 CFU/mL respectively. All samples for the LoD study were prepared in pooled and screened Strep negative clinical throat swab specimens and tested independently. The study was performed on a N288 NeuMoDx Molecular System and a N96 NeuMoDx Molecular System. Variant strains were prepared in neat Liquid Amies and 5 replicates of each strain were tested until a hit-rate of 100% was observed.

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

tion	<i>Streptococcus</i> Group	
	Group A	
sp.	Group C	
sp.	Group G	

Method Comparison

A total of 230 de-identified, retrospective residual throat swab specimens from symptomatic patients at two clinical laboratories (Beaumont General Hospital, Royal Oak, MI; Tampa General Hospital, Tampa Bay, FL) were tested on qualified NeuMoDx Molecular Systems. Results reported from the FDA/CE-IVD cleared tests (Quidel Lyra Direct Strep Assay [Beaumont] and Quidel Solana Complete Strep Assay [Tampa]) used by the clinical laboratories were utilized to perform the clinical performance analysis.

The NeuMoDx Strep A/C/G Vantage Assay had excellent concordance with the FDA/CE-IVD cleared tests, displaying a Clinical Sensitivity of 100% for the GAS target, Clinical Sensitivity of 95.9% for the GCS/GGS target, and Clinical Specificity of 100% for both targets.

Clinical Throat Swabs	# Swabs	Reference L
GAS Positive	68	Beaumont G Roval Oak, N
GCS/GGS Positive	49	Direct Strep
GAS/GCS/GGS Negative	113	Tampa Gene
TOTAL	230	Tampa Bay, Complete St

		Reference Lab		
	GAS	Positive	Negative	Total
Dx	Positive	68	0	68
Mo	Negative	0	162	162
Ne	Total	68	162	230
	GAS sensitivity: 68/68 = 100% (95% CI 93.3%-100%)			
	GAS specificity: 162/162 = 100% (95% CI 97.1%-100%)			

specificity: 102/102 - 100% (95/

		Reference Lab		
	GCS/GGS	Positive	Negative	
Dx	Positive	47	0	
Mo Mu	Negative	2	181	
Sei	Total	49	181	
L	GCS/GGS sensitivity: 47/49 = 95.9% (95% CI 84.9% - 99.3%)			9.3%)

GCS/GGS specificity: 181/181 = 100% (95% CI 97.4%-100%)



CONCLUSION The NeuMoDx Strep A/C/G Vantage Assay implemented on the NeuMoDx Molecular Systems is the first and only fully automated test developed for direct detection of Group A and Group C/C Streptococcus size literature to the test of the second streptococcus size literature to the second streptococcus of Group A and Group C/C Streptococcus size literature to the second streptococcus of Group A and Group A a developed for direct detection of Group A and Group C/G Streptococcus simultaneously from throat swab specimens. With ultra-fast turnaround time, minimum user intervention and high throughput, the NeuMoDx Strep A/C/G Vantage Assay addresses many unmet market needs.



- 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: ~50 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

_ab (Test): General Hospital,

MI (Quidel Lyra Assay) neral Hospital, FL (Quidel Solana

Strep Assay)

Analytical Specificity and Commensals

No cross-reactivity or interference was detected for any of the following 45 phylogenetically similar or co-habiting non-target organisms when tested with the NeuMoDx Strep A/C/G Vantage Assay. This resulted in an Analytical Specificity of 100%.

Pathogen Name	Final Concentration	Pathogen Name	Final Concentratio	
Stenotrophomonas maltophilia			10	
Klebsiella pneumonia	0.02-0.03	Parainfluenza Type 4b	ng/mL	
Acinetobacter Iwoffii	McFarland unit/mL	Corvnebacterium dinhtheria		
Streptococcus agalactiae		Strentococcus oralis	-	
Lactobacillus acidophilus		Peptostreptococcus micros (aka	0.02-0.03	
	1F6	Parvimonas micra)	McFarland unit/mL	
Adenovirus Type I	TCID50/mL	Enterococcus faecalis		
Streptococcus anginosus		Streptococcus pneumoniae		
L'actococcus lactis	-	Pseudomonas aeruginosa		
Strentococcus hovis	0.02-0.03	Escherichia coli	0.02-0.03	
Logionalla micdadai	McFarland	Streptococcus salivarius	McFarland	
	unif/mL	Fusobacterium necrophorum	unit/mL	
Arcanopacterium naemolyticum		Streptococcus sanguinis	•	
Streptococcus canis			1F6	
Legionella pneumophila	-	Rhinovirus Type 1A	c/mL*	
Bacillus cereus	0.02-0.03	Serratia marcescens		
Streptococcus gordonii	McFarland unit/ml	Haemonhilus influenzae type A	0.02-0.03	
Moraxella cartarrhalis		Strontococcus suis	McFarland	
Streptococcus intermedius		Stepholoccus suis		
Bordetella pertussis	10 ng/mL	Influenza A	1E6 c/mL	
Neisseria gonorrhoeae			156	
Neisseria subflava		Influenza B	c/mL	
Burkholderia cepacia	0.02-0.03	Streptococcus equi subsp.		
Streptococcus mitis	McFarland unit/mL	zooepidemicus (group C)	0.02-0.03	
Candida albicans		Staphylococcus epidermidis (MSRE)	McFarland unit/mL	
Streptococcus mutans		Streptococcus canis		

On board storage of room temperature stable reagents enables easy on demand operation.



Interfering Substances

The performance of the NeuMoDx Strep A/C/G Vantage Assay in the presence of potentially interfering substances that may be associated with collection of a throat swab from a patient were assessed on the NeuMoDx Molecular System. Neat Liquid Amies spiked with 150 CFU/mL Group A Strep, 7,500 CFU/mL of Group C Strep and 30,000 CFU/mL of Group G Strep was dosed with endogenous and exogenous moieties dissolved or diluted in molecular grade water at the specified concentration utilizing a saturated swab. None of the listed substances had an adverse effect on the detection of GAS, GCS or GGS using the NeuMoDx Strep A/C/G Vantage Assay on the NeuMoDx Molecular System.

Potentially Interfering Exogenous Substances	Stock Solution Concentration
Altoids – Spearmint Flavor	10% w/v
Aspirin	10% w/v
Icebreakers Mints- Cool Mint Flavor	10% w/v
Cepacol Extra Strength Sore Throat & Cough Lozenges	5% w/v
Chloraseptic Sore Throat Spray	10% v/v
Children's Dimetapp Cold & Cough	15% v/v
Crest Pro-Health Advanced Gum Protection	4% w/v
Cold-Eeze Zinc Lozenges	15% w/v
Halls Menthol-Lyptus Cough Drops	15% w/v
Halls Cherry Flavor Cough Drops	15% w/v
Listerine Ultra-clean Antiseptic Mouthwash	15% v/v
Listerine Total Care Mouthwash	15% v/v
Ricola Original Swiss Sugar Free Herb Cough Suppressant Throat Drops	15% w/v
Wal-Tussin DM Max Cough Syrup	10% v/v
Robitussin Max Strength Nighttime Cough DM	10% v/v
Sucrets Sore Throat & Cough Lozenges- Vapor Cherry	5% w/v
Tic Tac Freshmints	10% v/v
Chloraseptic max Lozenges	10% w/v
Potentially Interfering Endogenous Substances	Stock Solution Concentration
Whole Blood	10% v/v
Saliva	100%

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